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Resistance of Anhydrobiotic Aphelenchus avenae to Methyl Bromide Fumigation

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Abstract: The effect of methyl bromide (MB) was tested on active and anhydrobiotic *Aphelenchus avenae*. *A. avenae* was induced into anhydrobiosis by three different techniques. Both active and anhydrobiotic nematodes were subjected to 3,000 μl MB/liter air for 14 periods from 0 to 82 h. Anhydrobiotic nematodes were more resistant to fumigation than active nematodes, regardless of the technique used to induce anhydrobiosis. The percent survival decreased with increasing MB exposures (μl MB × h). For an LD_{95} of 45,000–54,000 $\mu l/l \times h$ were required for active nematodes and >279,000 $\mu l/l \times h$ for anhydrobiotic nematodes.

Key Words: anhydrobiosis, *Aphelenchus avenae*, survival, methyl bromide, soil fumigation.

Fumigation of soils for control of nema- odes is rarely eradicative. In addition to ysicochemical factors, other factors may responsible for poor control. Biological ctors, such as differences in susceptibility species and races of organisms, ecological abitat of nematodes in soil niches, or stage life cycle, are extremely important. Soil ematodes can enter a survival state or yptobiosis at any stage in their life cycles hen environmental conditions are stressful . They can exist in this state for long eriods and survive temperatures as low as 196 C and/or a relative humidity of 0% ith P_2O_5. Kostuk (13) suggested that soil ematodes in this survival state would be ore resistant to pesticides. Cooper *et al.* induced nonfeeding *Aphelenchus avenae* dults into cryptobiosis in a pure N_2 envi- nment and found 100% survival after 12 in 1,000 ppm EDB (1, 2-dibromoethane), hile only 35% survived as active nematodes under aerobic conditions. Anhydrobiosis, a form of cryptobiosis induced by dehydration, is a common phenomenon in soil nematodes (4). This study was done to test the effect of methyl bromide (MB) on anhydrobiotic *A. avenae*, a fungivorous nematode.

MATERIALS AND METHODS

Aphelenchus avenae Bastian, 1865, was cultured in the laboratory on *Rhizoctonia solani* Kuhn, 1858 (2, 10). Active nematodes, L_4 and adults, were removed from the sides of culture jars by rinsing the walls of the jars with distilled water and centrifuging the water-nematode suspension at 3,000 rpm to concentrate the nematodes. The nematodes in the precipitate were washed twice and collected on a 26-μm (500-mesh) sieve. Anhydrobiosis was induced by three techniques. With Technique I, following Crowe and Madin (5), samples of moist active *A. avenae* weighing 0.1 g each were slowly desiccated for 3 days in chambers designed to maintain a relative humidity of 97%. The mass of coiled anhydrobiotic nematodes (pellets) was placed over P_2O_5 (0% humidity) for 3 days, removed, and cut into small pieces (about 0.0125 g), and each piece was mixed into 100 ml dry sandy loam soil (72.8% sand, 21.2% silt, 6.0% clay) in 250-ml Erlenmyer flasks. Percent of active

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nematodes was determined for the smaller pieces of the pellet after 24 h by bubbling them in tap water.

With Technique II, following Demeure *et al.* (6), active *A. avenae* were induced into anhydrobiosis by placing them in saturated sandy loam soil on a pressure plate [5-bar pressure-plate extractor (Cat. No. 1600); Soil Moisture Co., Santa Barbara, California] and slowly increasing the pressure to 3 bars [about 2.5% (w/w)] soil moisture, at which time 98% of the nematodes were in the anhydrobiotic state. As with Technique I, anhydrobiotic nematodes were placed in 100 ml of soil in 250-ml flasks.

With Technique III, following Simons (6) as modified by Demeure *et al.* (7), anhydrobiosis was induced in small quantities of nematodes on a Millipore (100 ± 5) filter (diam 13 mm; aperture 0.6 μm, Millipore Corporation, Bedford, Massachusetts, 01730, Cat. No. SSWP 01300) by slow desiccation in a relative-humidity chamber using a modification of Simon's dehydration schedule. One hour before fumigation with MB, the Millipore filters were suspended on a steel rod in 250-ml Erlenmyer flasks so they would not be in contact with the soil.

Soil in half of the flasks was moistened (9% w/w) 24 h before the experiment, allowing the nematodes induced into anhydrobiosis by Techniques I and II to become active before treatment with MB. For Technique III, active nematodes were placed on a moistened Millipore filter. The other half of the flasks contained anhydrobiotic nematodes in dry soil (2.5% w/w) and on dry Millipore filters. The flasks containing soil and Millipore filters were attached to the manifold of an MB fumigation apparatus that gave a continuous flow of MB gas (20 ml/min) at a constant concentration (12). The concentration of MB in each experiment as it exited from the flasks was monitored frequently with a gas chromatograph. The methyl bromide-air mixture was dried by passage through Drierite to assure that no moisture entered the system to activate the anhydrobiotic nematodes, then passed through the soil, and finally evacuated through an exit tube at the top of the flask. The treatment of active nematodes was similar except that MB was bubbled through water to assure that no drying occurred in the moist soil. Controls were identical to MB treatments except that air only (moist or dry) was passed through the soil. The nematodes were subjected to a concentration of 3000 μl MB/liter for 14 periods from 0 to 82 h. Concentration × time (CT) values were calculated by multiplying concentration of MB × h of exposure. For each treatment, three replicate experiments of Technique I and one replicate experiment each of Techniques II and III were treated at each CT.

After treatment for the appropriate number of hours, the soil was poured from the flasks onto paper in a hood for 15–30 sec to dissipate the MB gas, and placed in a plastic bag until all treatments were completed. Nematodes from Techniques I and II were extracted from soil by the anhydrobiotic nematode technique (11), and the percent of coiled nematodes in the sugar solution was determined. A tightly coiled morphological shape is characteristic of anhydrobiotic nematodes. The nematodes in the sugar solution were rinsed and placed in continuously aerated tap water for 24 h to allow the anhydrobiotic nematodes to rehydrate, and the active nematodes were counted. The criterion for activity was motility. Percent activity for anhydrobiotic nematodes on Millipore filters was determined by placing the filters in bubbling H_2O for 24 h.

RESULTS

The anhydrobiotic *A. avenae* were more resistant to MB than the active *A. avenae* (Fig. 1). The percent survival of active nematodes decreased rapidly with increasing MB exposures i.e., LD_{95} = 15 h, 18 h, and 18 h (CT = 45,000, 54,000, and 54,000) for Techniques I, II, and III, respectively. No active nematodes survived after 22 h (CT = 66,000). The LD_{95} for anhydrobiotic nematodes from Techniques I, II, and III was 93 h (CT = >279,000). Individual nematodes induced into anhydrobiosis by the Millipore-filter technique (Technique III) were the most resistant to fumigation, even though there was more variation than in soil. The correlation coefficients between CT and percent survival were respectively 0.961, 0.915, and 0.603 for Techniques I, II, and III.

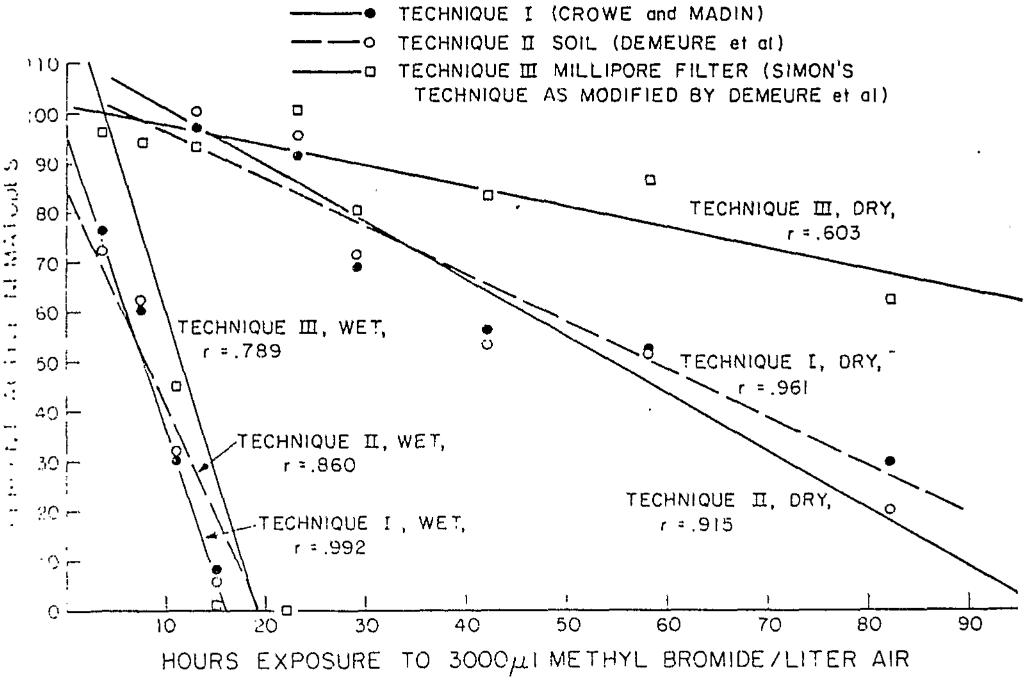

FIG. 1. Effect of methyl bromide at a concentration of 3000 µl/liter air upon the survival of active and anhydrobiotic *Aphelenchus avenae*.

DISCUSSION

The anhydrobiotic nematodes were more resistant to fumigation than the active nematodes, regardless of the technique used to induce anhydrobiosis. It required 45,000-54,000 CT (µl MB • l) to obtain an LD_{95} dose for moist active *A. avenae*, while the exposure had to be increased to five times (279,000 CT) to obtain the same kill of anhydrobiotic nematodes. Comparisons of LD_{95} data indicate that active *A. avenae* was more resistant to MB than other active nematode species (Table 1), and that the amount of chemical necessary for an LD_{95} with anhydrobiotic *A. avenae* was about 17 times as great as for an LD_{95} with active *Xiphinema index* and *Meloidogyne incognita* (17). It is possible that the anhydrobiotic nematodes might tolerate lower CT levels and that achieving $>LD_{95}$ would require a greater concentration of MB. Vannecke et al. (unpublished data) [illegible] a similar situation with sclerotia of *Sclerotium rolfsii* Sacc. and microsclerotia of *Verticillium albo-atrum*. Similar information could be obtained from the field with [illegible] relative humidity and moisture ranges as suggested by McKenry (15). Since MB concentrations do not remain as high and as constant in the soil as they were in this experiment, anhydrobiotic nematodes could be expected to survive and serve as a new source of inoculum. Recent studies (6) have shown that the number of soil nematode species capable of entering anhydrobiosis is much larger than previously thought and includes many plant parasites, i.e., *Helicotylenchus dihystera, Meloidogyne* spp., and *Scutellonema cavenessi*.

TABLE 1. Concentration × time (CT) exposure to methyl bromide required for LD_{95} in different nematode species.

Nematode	CT in µl × h	Reference
Meloidogyne incognita larvae	16,800	17
Xiphinema index adults	16,200	17
Dorylaimus sp.	21,000	17
Xiphinema index larvae	23,400	17
Aphelenchus avenae larvae and adults	45,000-54,000	This report
Anhydrobiotic *A. avenae* larvae and adults	>279,000	This report

The resistance of anhydrobiotic *A. avenae* to MB needs to be examined in more detail. A basic principle of biology is that water is needed for the maintenance of biological integrity. Anhydrobiotic nematodes have a total water content of only 0–5% (5, 8, 9), an undetectable metabolism, and a morphological form that has changed the nematode internally and externally from a hydrated eel-like form to a tightly shrunken coil without irreversibly damaging the nematodes. These facts lead us to suggest three possibilities for the resistance of the anhydrobiotic nematode to MB: 1) MB fails to permeate the nematode cuticle because of structural and physiological changes in the anhydrobiote; 2) the MB permeates the cuticle, but is not reactive with the lowered metabolic processes; and 3) MB is soluble in water, and therefore the MB concentration is greater surrounding the active nematodes in moist soil than around the anhydrobiotic nematodes in dry soil. Concerning the last hypothesis, it has been shown that methyl bromide has an affinity for water and that an equivalent volume of MB is more highly concentrated in water than in air in the gaseous phase (1). For this reason the CT calculations for the active nematodes in moist soil may not be accurate and the anhydrobiotic nematodes may be more susceptible to the chemical than indicated by these tests. On the other hand, Marks et al. (14) found that the dynamic equilibrium of EDB between five different nematodes and the bathing solution was reached after about a 30-min exposure and that the concentration inside the nematodes was 2–20 times that in the bathing solution. This suggests that, to be toxic, the chemical must get into the organism in greater quantities than in the bathing solution. This would lend support to hypothesis 1 or 2, or a combination of the two.

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