Dosage Response of Armillaria mellea to Methyl Bromide

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ABSTRACT

Citrus root pieces artificially infested with *Armillaria mellea* were subjected to several doses of methyl bromide gas for various periods of time, aerated, and buried in nonsterile soil for 21 days. After storage, the inoculum pieces were sampled for viability of *A. mellea* by plating-out chips taken from the roots on agar medium. The LD_{95} values were determined for various conditions and fitted to a standard curve. The LD_{95} values ranged from 1.6 days at 3,000 ppm methyl bromide in air to 9.5 days at 500 ppm. Phytopathology 60:992-993.

Armillaria mellea (Vahl.) Quel. is capable of dormant existence deep in soils on dead roots or stumps of trees and shrubs for many years. Although experimental data are lacking on the depth in soil at which *A. mellea* may exist, observations in California and elsewhere indicate that the fungus may penetrate as deep as the host roots. Consequently, in our experiments on the use of fumigants for controlling *A. mellea,* we are investigating means to facilitate penetration of methyl bromide to depths of over 3 m. Naturally, the concentration of methyl bromide decreases with increase of the distance from the site of application, and concentrations deep in soil may be quite low (3). Yet, the low concentration may persist for long periods of time, so it is imperative to have an estimate of the dosage response of *A. mellea* based upon concentration of methyl bromide as well as upon the time of exposure of the fungus to the gas.

To determine the dosage response of *A. mellea* to methyl bromide in the laboratory, several unique problems exist. Although the basidiospores of the fungus are viable and readily produce mycelial colonies in culture media, they apparently are incapable of infecting a host. Therefore, the spores were not used. Also, *A. mellea* persists in soil in intimate association with its host, never growing much into the soil unless anchored to a rather large root or stem that serves as a reservoir food source. For these reasons we chose to use rather large pieces of roots heavily infested with *A. mellea* mycelia as test material. This severely limited the number and units available for individual treatments and replications.

In addition to the primary lethal effect of the toxicant on the fungus, there is a biological effect that enhances the fungicidal effectiveness of the gas. Much lower concentrations of toxicants are effective in killing *A. mellea* in the presence of soil and its associated organisms than for outright kill of pure cultures. This effect had to be considered in devising a suitable assay technique.

Our purpose was to devise techniques for determining a dosage response of *A. mellea* to methyl bromide that reasonably could be utilized to evaluate field fumigations.

MATERIALS AND METHODS.—*Preparation of inoculum.*—Inoculum prepared from 1963-69 varied slightly from one experiment to the next. It consisted of whole citrus root pieces 12-15 cm long and 2.5-3.5 cm in diam. From three to five root pieces in 2-liter jars, each containing 200 ml water, were sterilized by heating for 1 hr at 110 C on 2 successive days.

Isolate D-73 (isolated from infested citrus roots at Redlands, Calif.) was grown in petri dishes. The medium consisted of 200 g of wood prepared by drying citrus sucker growth and grinding it sufficiently fine to pass a 7-mm screen. The wood was combined with 20 g sucrose, 15 g agar, 0.01 g pentachloronitrobenzene, and 1 liter water. After it was cooked for 1.5 hr, the medium was strained through cheesecloth and sterilized by holding for 30 min at 110 C. After the fungus had grown on the agar medium for approximately 4 weeks, the mycelia and rhizomorphs were peeled off, cut into pieces 1 cm^2, and scattered over the root pieces in the jars. The jars were covered with a screw cap in which a hole of 1 cm diam was cut to facilitate exchange of air. The hole was plugged with cotton and the jars stored at 20 C. Mycelial growth was profuse over the roots after a few months. The pieces were used within 1 year, although a few experiments were made with inoculum that was either 6- or 24-months old. Before exposing the infected root piece, the outside layers of mycelium and rhizomorphs were trimmed off. The roots were thoroughly infested, although the wood remained firm. Tests showed that *A. mellea* was viable in the center as well as in the outer portion of the roots. Efforts were made to obtain inoculum of uniform size and condition for all treatments. Before fumigating, the inoculum pieces were moistened with water and rolled in freshly collected citrus orchard soil so that each piece was covered with soil.

Treatment with methyl bromide.—Four to six pieces of inoculated roots were placed on a wire mesh stand in a 2-liter jar so that they were held approximately 2 cm from the bottom. Methyl bromide in air of the desired concentration was introduced at the bottom of the jar beneath the mesh stand, and escaped through an outlet at the top of a special gas-proof cap.

The desired concentrations of methyl bromide in air were prepared by mixing pure methyl bromide and compressed air streams and passing the mixture through a manifold. Each outlet arm of the manifold had a restrictor to regulate the flow at 20 ml/min, the input rate to each jar during fumigation. The concentrations at the entrance and exit ports of the jars were monitored at least daily by gas chromatography (M. J. Kolbezen, *personal communication*). Three concentra-

tions of methyl bromide (approximately 550, 1,100, and 2,200 ppm) for each experiment were applied continuously for 1 to 16 days. Controls were identical to the methyl bromide treatments except that air only was passed over the inoculum for the longest time of the experiment.

Postfumigation treatment.—After treating them with methyl bromide, the inoculum pieces were aerated for several hr, placed in another 2-liter jar, covered with unsterilized citrus orchard soil, and stored for 21 days at 20-25 C. Each treatment was held in a separate jar.

Evaluation of treatment.—After the postfumigation treatment, two to four small chips of wood aseptically removed from the inner portions of inoculum pieces were tested for viability by placing them on citrus agar in test tubes. The tubes were observed regularly for 30 days; if viable, growth of *A. mellea* was visible usually in 10 days. The presence of other organisms, usually *Trichoderma viride* Pers. ex Fr., was frequently observed, especially from pieces having no viable *A. mellea*. The per cent nonviable *A. mellea* pieces was determined and used to plot dosage response curves.

RESULTS.—From 1964-69, 12 experiments were made to determine the dosage response of *A. mellea* to methyl bromide. Because of the variable material, no experiment was an exact duplicate of another. Dosage response curves for each experiment were plotted on a graph for each dose applied using per cent kill of *A. mellea* vs log days of exposure to the gas as variables. It was apparent that the curves fell into 3 groups corresponding approximately to the following concentrations of methyl bromide: 400-600 ppm, 800-1,200 ppm, and 1,500-2,200 ppm. Dosage response curves from several experiments were chosen as being most representative of each of the three groups. The LD_{95} values were determined for each curve so selected. These LD_{95} values are presented in Fig. 1. In the figure, the log days of exposure are plotted against the log concentration of methyl bromide necessary to result in a LD_{95} effect for that period. The LD_{95} ranges from approximately 9.5 days at 500 ppm, and by extrapolation, to approximately 1.6 days at 3,000 ppm. Fumigations at concentrations below 400 ppm gave erratic results.

DISCUSSION.—The data substantiate the proposition that if an infested root or stem were subjected to certain combinations of time/concentration of methyl bromide (as determined from Fig. 1) and left in soil for at least 21 days after fumigation, 95% of *A. mellea* would be killed. Caution should be exercised, however, since extrapolation to the field is only an approximation, and also the curve is based upon a LD_{95} value. Prediction of eradicative doses is nearly impossible, especially in the field. Hence, we have settled on the LD_{95} value. As a result, we are reasonably confident that these values are applicable to most field experiences.

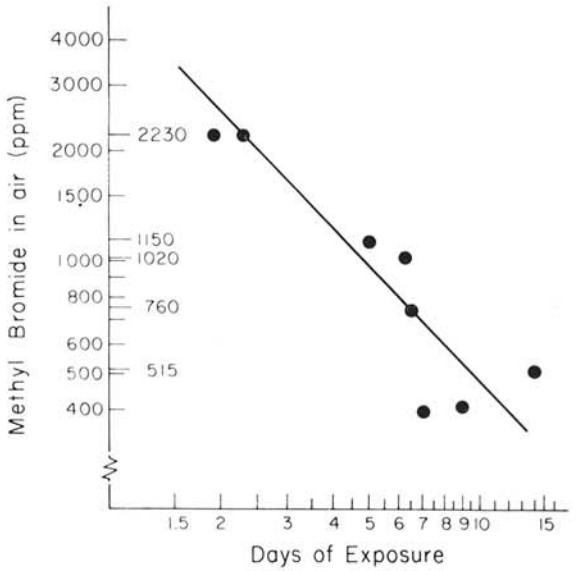

Fig. 1. Relationship of days of exposure and concn of methyl bromide necessary to result in a LD_{95} of *Armillaria mellea* growing in citrus root pieces. Inoculum was stored 21 days in nonsterile soil after fumigation before sampling for viability.

These experiments are a part of a long term continuing research project at the Univ. of Calif., Riverside, initiated by Bliss (1) in the early 1940's. We currently are determining by chemical analysis the concentration of methyl bromide in field soil atmospheres at various depths when applied by several techniques (2, 3). We hope to combine these laboratory data with actual measurements of methyl bromide so that more accurate control measures may be formulated.

LITERATURE CITED

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