Research Note

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EFFECT OF WATER ON THE CONCENTRATION OF CYCLOHEXIMIDE IN FUEL OIL

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ABSTRACT

Water removes cycloheximide from oil solvent, but the degree of removal could not be determined because neither the oil nor the water fraction containing cycloheximide could be bioassayed directly.

INTRODUCTION

The poor results sometimes obtained when treating western white pine trees with the antibiotic cycloheximide to control blister rust have been attributed to two physical factors: [2]

1. Incompatibility of cycloheximide with some brands of oil.

   This problem has been solved.

2. Affinity of cycloheximide for water.

   It was suggested that water in equipment used for spraying the oil solution of cycloheximide extracts enough of the antibiotic from the oil to seriously lower the dosage of the antibiotic applied to trees.

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[2] Moss, V. D., T. R. Peterson, and W. E. Bousfield. Antibiotic development and improvement work. IN: White Pine Blister Rust Control, Calendar Year 1960. U.S. Dept. Agr., Forest Service, Region One, Missoula, Mont., pp. 38-49. 1961.

Therefore it seemed desirable to determine how much cycloheximide is thus removed from the oil so that enough antibiotic might be added to compensate for this loss.

METHODS

Cycloheximide was first dissolved in 10 ml. of acetone. This cycloheximide solution was added to No. 1 stove oil containing 0.2 percent Triton 1956B to obtain solutions of 150, 200, and 250 p.p.m. of antibiotic. Distilled water was added to aliquots of each concentration of the solutions to make up the following percentages of water by volume: 0.0, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0. The mixtures were then stirred for 10 minutes at room temperature on a Fisher Thermix[3] at its highest speed setting. Each emulsion was centrifuged at 15,000 times gravity for 15 minutes in a refrigerated centrifuge at 20° C. The upper portion of the oil phase was saved; the lower portion of the oil phase and all the water were discarded. Oil containing 0.2 percent Triton 1956B was used to make subsequent dilutions. A twentyfold dilution was then bioassayed by Whiffen's method.[4] A sample (0.07 ml.) of each solution was applied directly to 12 paper discs (4 discs per plate).

RESULTS AND DISCUSSION

We originally planned to bioassay all fractions of the oil-water mixture, but it was obvious that not quite all the oil had been removed from the water by centrifuging because the water was tinged faintly but distinctly yellow. Since even high-speed centrifuging did not remove all the oil from the water phase, and since the amount of oil left in the water appeared to vary from sample to sample, the water fraction was not used in the bioassay.

When bioassays using water as the solvent for cycloheximide were carried out, the resulting curves were similar to Whiffen's. These curves could not be obtained when oil containing Triton 1956B was used as the solvent. Failure to obtain suitable bioassay curves from oil solutions was established by statistical analysis of the bioassay curves in figure 1; no significant differences were detected among the three concentrations of cycloheximide.[5]

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[3] Use of trade name for equipment is solely for identification and does not imply endorsement or recommendation by the Forest Service.

[4] Whiffen, A. J. The production, assay, and antibiotic activity of Acti-dione, an antibiotic from Streptomyces griseus. Jour. Bact. 56: 283. 1948.

[5] Statistical analyses by M. A. Marsden, Statistician, Intermountain Forest and Range Experiment Station, Moscow, Idaho.

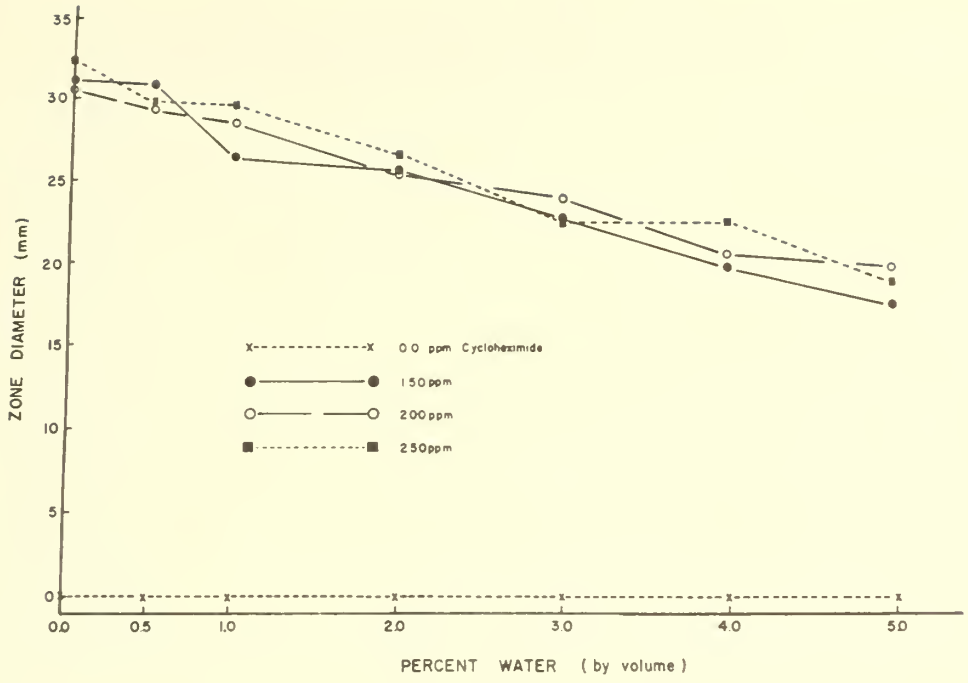

Figure 1.--Inhibition of Saccharomyces pastorianus by oil solutions of cycloheximide after extraction with water. Zone sizes include the 12.7 mm. paper disc except for the 0.0 p.p.m. antibiotic solutions, which produced no inhibition zone under the disc.

Although statistical analysis of the data does not show clear separation among the average zone diameters for the three concentrations of the antibiotic, the slope of the regression of zone diameter on percent water was significantly different from zero; i.e., the more water that was in contact with the oil solution of cycloheximide, the more the concentration of the antibiotic in the oil was reduced. Also, the zone diameters at the average concentration of water do fall in the expected sequence according to concentration (fig. 1). The lack of discrimination may be the result of combining a response error due to the water treatment with the inherent variation of the bioassay itself.

Two conclusions are reached from the results of our study.

1. This bioassay procedure using oil containing Triton cannot be used to determine the concentration of cycloheximide.

2. Although we were not able to recommend the amount of antibiotic to be added to compensate, the fact remains that cycloheximide is removed from oil by water, as was originally suggested.[6]

[6] Moss, et al., op. cit., p. 1.