Increased Sensitivity to Zineb for Verticillium malthousei Strains Tolerant to Benomyl

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ABSTRACT

Conidia of four isolates of *Verticillium malthousei* tolerant of benomyl were more sensitive to zineb solutions ranging to 20 μg/ml than were conidia from four isolates sensitive to benomyl. Equivalent reduction in spore germination and germ tube length occurred at zineb concentrations half those required for the strains sensitive to benomyl.

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Additional key words: fungicide effectiveness, mushroom disease.

Verticillium malthousei Ware is the causal agent of dry bubble disease of the commercial mushroom *Agaricus bisporus*. The standard fungicides used for control of this disease are zineb (zinc ethylene bisdithiocarbamate) and benomyl [methyl 1 - (butylcarbamoyl) - 2 - benzimidazolecarbamate] (2). However, tolerance of benomyl by this pathogen developed within 1 year of its initial use, and is now common. A general discussion of benomyl tolerance is available in reference (1). Upon inquiry, one mushroom grower reported increased effectiveness of zineb following loss of control by benomyl (W. Gerner, Butler County Mushroom Farm, West Winfield, Pa.; *personal communication*, 12 March 1974). This study was undertaken to determine if an alteration in sensitivity to zineb has been a general consequence of the increased occurrences of benomyl-tolerant isolates in the fungal population.

Eight strains of *V. malthousei* were used; four isolated in 1973 were tolerant of benomyl, and four isolated before 1972 were sensitive to benomyl. Each was received from a different location. One million conidia, washed from 10-day-old Czapek Dox agar cultures, were added to zineb (Parzate 75% WP) solutions containing either 0, 1, 5, 10, or 20 μg/ml (a.i.) zineb in a test tube containing 10 ml of distilled water. After 24 hours of incubation at 20 C, 1 ml lactophenol-cotton blue was added to each test tube to fix and stain the spores. Germination and germ tube length for 25 spores was recorded for each of three replicates. The average percent germination and germ tube length of the four tolerant strains was compared statistically to the four sensitive strains by combining data from each group of four strains and analyzing them by Student's *t*-test at each zineb concentration.

At 0 and 1 μg/ml zineb, average germination for all strains exceeded 95% (Fig. 1) and there was no statistically significant difference in germ tube length between sensitive and tolerant strains (Fig. 2). At higher concentrations, zineb reduced spore germination and germ tube elongation of the benomyl-tolerant isolates more effectively than with the benomyl-sensitive isolates. The effectiveness of zineb on the benomyl-tolerant strains

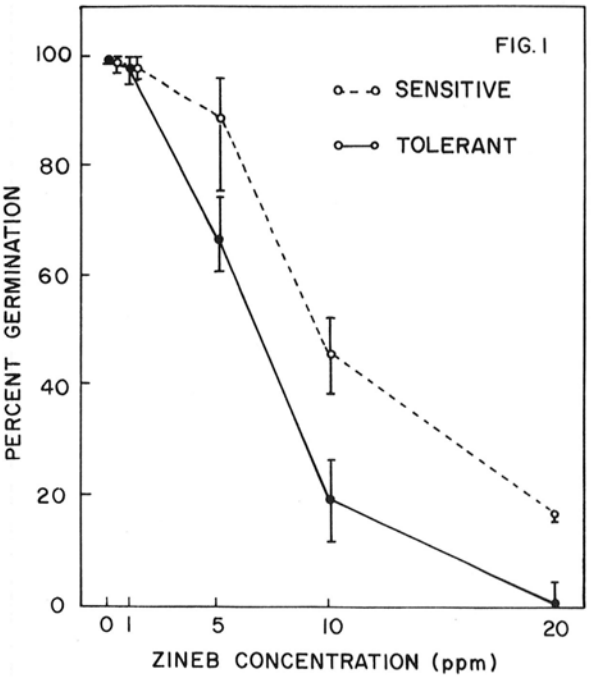

Fig. 1. Average percentage spore germination of four *Verticillium malthousei* isolates tolerant of benomyl and four benomyl-sensitive isolates following 24 hours of incubation in zineb. Brackets indicate range of each set of isolates.

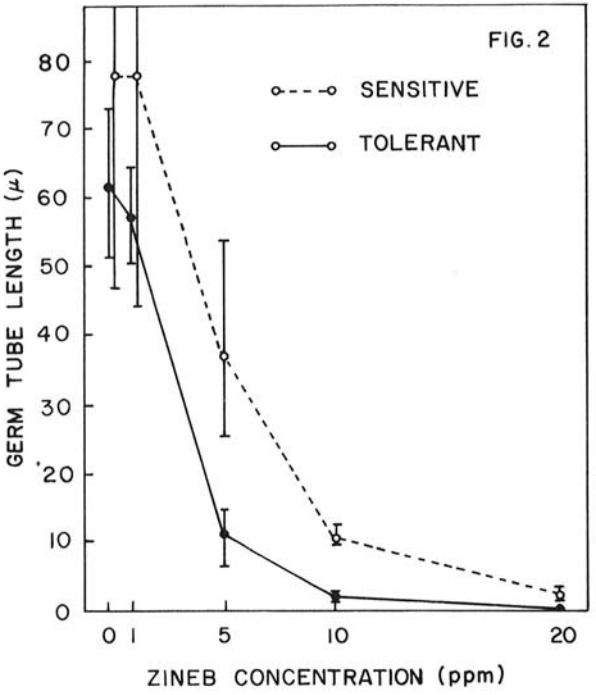

Fig. 2. Average germ tube length of four *Verticillium malthousei* isolates tolerant of benomyl and four benomyl-sensitive isolates following 24 hours of incubation in zineb. Brackets indicate the range of each set of isolates.

at 5 and 10 μg/ml was similar to that of 10 and 20 μg/ml zineb, respectively, on the benomyl-sensitive strains for both germination and germ tube length. Differences in germination and germ tube length between tolerant and sensitive strains were significant ($P = 0.01$) for treatments with $\geqslant 5$ μg/ml zineb.

For *V. malthousei*, a consequence of tolerance to benomyl has been increased sensitivity to zineb. There are two other recent reports of altered sensitivity to protectant fungicides in benomyl-tolerant strains, although only single isolates were compared (3, 4). Should the mechanism of benomyl tolerance in other fungal species be similar to that for *V. malthousei*, the response of these species to other fungicides may be affected. Increased sensitivity to fungicides in plant pathogenic fungi tolerant of benomyl and other fungicides may be of wider occurrence than presently anticipated and may significantly influence the persistance of these tolerant strains in the field.

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