Suppression of Dutch Elm Disease in American Elm Seedlings by Benomyl

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The authors thank the E. I. duPont de Nemours Co., for supplying the Benomyl fungicide, E. J. Koch for the statistical analysis, and D. E. Wuertz for technical assistance.

For many years, investigators have sought chemotherapeutic materials for the control of Dutch elm disease (D.E.D.). Unfortunately, all efforts have been unsuccessful to date. The recent discovery and development of several systemic fungicides (1, 3, 4, 5, 9, 10) have given new impetus to the search for chemotherapeutic agents that will control D.E.D.

This note reports evidence for the systemic fungitoxicity of methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (Benomyl), based on the suppression of D.E.D. symptoms and the failure to isolate *Ceratocystis ulmi* (Buism.) C. Moreau from treated, inoculated American elm (*Ulmus americana* L.) seedlings.

Dormant 1-, 2-, and 3-yr-old elm seedlings were planted in 8-inch plastic containers in white silica sand and grown in environmental chambers under a 19-hr photoperiod (1,800 ft-c combined incandescent, cool-white fluorescent light) and a constant thermoperiod (27 C day, 19 C night). Weekly applications of 200 ml of a modified Hoagland's solution (8) were applied to each container when the buds began to swell. When the leaves were fully expanded, two groups of 10 plants each from each of the three age groups were treated twice a week with 200 ml of either a 500-ppm or a 1,500-ppm (active ingredient) aqueous suspension of Benomyl applied as a sand drench. These concentrations were selected on the basis of preliminary studies which indicated that concentrations of Benomyl greater than 2,000 ppm were phytotoxic to the seedlings. Appropriate water checks were provided.

After four applications of Benomyl, each tree was inoculated (6) with 3 ml of a conidial suspension of *C. ulmi* standardized to an optical density (OD) of 0.06 at 450 mμ (7). Seedlings then received eight additional Benomyl treatments. Five days after the final treatment, they were examined for foliar symptoms of D.E.D., cut off at the sand line, and divided into three regions: (i) top (characterized by fully expanded terminal leaves; (ii) center; and (iii) bottom (50 mm above sand). Leaf discs (13-mm diam) and stem sections (25 mm long) from each region were placed in plastic petri dishes containing 12-14 ml potato-dextrose agar (PDA) which had been seeded with 1 ml of a standardized conidial suspension of *C. ulmi* (OD 0.08 at 450 mμ) per 100 ml PDA. After the plates were incubated at room temperature for 48 hr, zones of inhibition around plant tissues were measured to determine the relative concentration of inhibitory substances present in the seedlings. Stem sections adjacent to those used in the bioassay were placed on acidified PDA plates to determine the presence to *C. ulmi* in the wood.

Analyses of top-leaf and center-leaf from Benomyl-treated seedlings did not show significant differences in the sizes of the mean zones of inhibition between the two concentrations or between ages of trees (Table 1). However, they were significantly different from the untreated plants. For top-stem in 1-yr-old trees, a concentration of 1,500 ppm produced significantly larger zones of inhibition than did 500 ppm. No significant differences occurred in the sizes of the zones of inhibition between the two concentrations in either center-stem or bottom-stem within any age group. No definitive trends could be established between the ages of the plants and the amount of Benomyl accumulated in the tissues at either concentration. Benomyl or a Benomyl derivative (2) was readily absorbed from the sand and translocated throughout all the treated plants. We attempted to determine the approximate concentrations of the inhibitor in the tissues by means of in vitro

TABLE 1. Average diam of inhibition zones surrounding tissue sections from elm seedlings treated with Benomyl and from untreated seedlings[a]

Concn of Benomyl	Age of seedlings	Zones of inhibition (mm diam)				
		Top[b]		Center		Bottom
		Leaf	Stem	Leaf	Stem	Stem
ppm	*yr*					
0	1,2,3	0 b[c]	0 d	0 b	0 c	0 d
500	1	42 a	22 c	38 a	37 a	37 c
	2	38 a	32 ab	44 a	24 b	43 ab
	3	35 a	37 ab	47 a	40 a	45 ab
1500	1	36 a	38 a	46 a	34 ab	36 c
	2	39 a	29 bc	39 a	29 ab	48 a
	3	34 a	34 ab	42 a	38 a	41 bc

[a] Tissue sections incubated in petri dishes containing 12-14 ml potato-dextrose agar (PDA) which has been seeded with 1 ml of a standardized conidial suspension of *Ceratocystis ulmi* per 100 ml PDA and incubated at 22-25 C.

[b] Top, characterized by fully expanded terminal leaves; center, and bottom, 50 mm above sand.

[c] Means followed by the same letter are not significant at the 5% level according to Duncan's multiple range test and Kramer's method for unequal subclass numbers.

tests. Bioassay pads (13-mm diam) were saturated with various concentrations of Benomyl in 95% ethanol, air-dried, and placed in petri dishes on PDA which had been seeded with *C. ulmi* conidia. The zones of inhibition around the various tissues were comparable in size to those around bioassay pads which had been saturated in solutions of 10- to 40-ppm Benomyl.

The presence of *C. ulmi* in inoculated trees was determined by the appearance of foliar symptoms and its isolation from tissue. Forty-three per cent of the untreated plants had symptoms of D.E.D. as compared with 1.7% of the treated plants. The most striking contrast between treated and untreated trees was in the isolation of *C. ulmi*. We were unable to isolate *C. ulmi* from any of the 60 treated plants; whereas, we isolated the fungus from 80% of the check trees. Using both symptoms and recovery of the fungus as criteria, Benomyl showed marked fungitoxic activity.

These data strongly suggest that Benomyl warrants testing in the field for the control of D.E.D.

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