Control of Dutch Elm Disease in Artificially-Inoculated American Elms with Soil-Injected Benomyl, Captan, and Thiabendazole

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ABSTRACT

Benomyl, captan and thiabendazole at 124.8 g per tree each were injected under pressure into the Lodi loam rhizosphere of nine completely-randomized three-year-old American elms (*Ulmus americana*) prior to inoculation with *Ceratocystis ulmi*. Mean percent foliar symptoms after 14 months were 4, 23, 55 and 76 in benomyl-, thiabendazole- and captan-treated and control trees, respectively. Disease control in benomyl-treated trees was superior and highly uniform. Bioassays of solvent-extracted leaves from treated trees sampled 2 days after fungicide application revealed the presence of fungitoxicants. Vascular discoloration was present in at least half of the trees from each treatment, and biopsy tissues from benomyl-treated trees yielded the least number of *C. ulmi* cultures. Fresh weight of tops of manually-defoliated benomyl-treated trees was more than twice that of any other treatment, including fungus-inoculated, non-fungicide-treated controls. The surfactant adjuvant, Tween 80, used at 1% (v/v) with each fungicide did not appreciably affect disease control. Residual fungitoxicants were detected in all treated soils 17 months after application. Benomyl offers promise in controlling Dutch elm disease by soil injection prior to infection.

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Dutch elm disease (DED), the most serious malady of the American elm (*Ulmus americana* L.), destroys 400,000 trees per year in the U.S.A., a loss of $100 million (5). The recent ban on use of the vector insecticide, DDT (dichlorodiphenyltrichloroethane), has stimulated the search for alternate means of control. One of the most promising is the use of systemic fungicides.

The mobile nature of benomyl and its preventive or curative efficacy in controlling DED have been explored (1, 2, 4, 7, 8, 9, 12, 13). Trunk implantation consisting of packing a dry chemical into a drilled hole in the trunk or a branch has certain impractical and deleterious features (10), and recently has been found ineffective with benomyl (7). Hine et al. (2) demonstrated the immobile nature of benomyl in soil, and Hock & Schreiber (3) reported the inefficacy of passive soil placement of benomyl in the control of DED. Stipes (9) experienced limited success with dry-soil amendments in a sandy loam soil and Stipes & Oderwald (11) also reported the immobility of technical benomyl and thiabendazole on soil thin-layer chromatographic plates without the aid of surfactants (11).

In view of limited success if disease control by the previous methods, the soil injection route was evaluated. Use of this method avoids such objectionable factors as repeated mechanical disruption of bark and wood tissues, which provides infection courts for wood rotting organisms and immobilization of compounds. In addition to the soil injection of benomyl (1) thiabendazole and captan were included because they had shown efficacy in disease control by other application techniques (8, 9) but had never been soil injected. The enhancement of disease control of these fungicides plus the surfactant Tween 80 was also studied, since it was shown previously to be effective in moving fungicides on soil thin-layer chromatographic plates (11).

MATERIALS AND METHODS.—The American elms used in this study were planted as 2-year-old, 30 to 45 cm tall whips in 1969, 1.2 m apart in rows 1.2 m apart in Lodi loam at a nursery at Blacksburg, Va. Excavations of several root systems at the initiation of treatments in May 1970 revealed extensive proliferation at 0.31 m depth of laterals off the taproot that extended at least 0.62 m. Nine completely randomized trees per treatment were used.

The treatment zone, a 45-cm radius around the tree 30-cm deep, into which benomyl (50% WP), captan (50% WP) and thiabendazole (60% WP) were forcibly injected, received 6 liters of a 124.8 gm (active ingredient) suspension (ca. 1,962 kg/ha) with or without the surfactant (1.0% v/v) Tween 80. Soil injections were made 30 May 1970 using a pesticide sprayer which had the spray nozzle replaced with a soil probe. The rhizosphere of control trees received 1.0% Tween 80 or tap water.

Inoculum consisted of a washed suspension of 4.8 $\times 10^6$ conidia/ml derived from known pathogenic, composite isolates (cultures Va.-4, -6, -9, and -10) of *Ceratocystis ulmi* (Buism.) C. Moreau grown for 7 days on potato-dextrose agar (PDA). All trees were inoculated 12 June 1970 by permitting complete and rapid uptake of one drop of the suspension,

TABLE 1. Percent foliar symptoms of Dutch elm disease in artificially-inoculated American elms that received prior soil injections of benomyl, captan and thiabendazole, with and without the surfactant, Tween 80

Treatment[1]	Mean percent foliar symptoms								No. symptomatic trees	
	Days after inoculation									
	10	18	25	31	38	48	66	444[2]	66	444
Benomyl	0	0	<1	<1	<1	<1	<1	4	1	6
Benomyl + 1.0% Tween 80	7	11	7	6	7	6	6	9	3	7
Captan	28	67	67	74	78	79	78	55	9	9
Captan + 1.0% Tween 80	28	63	70	71	73	69	67	56	9	9
Thiabendazole	24	45	50	58	60	54	52	23	9	9
Thiabendazole + 1.0% Tween 80	15	49	47	50	52	47	44	28	9	9
Control, 1.0% Tween 80	34	54	60	61	64	66	67	54	9	9
Control, water	38	61	65	68	72	69	68	76	9	9

[1] Nine completely randomized trees per treatment.
[2] Means among all (1) fungicide alone and (2) fungicide + Tween 80 treatments were significantly different at the 1.0% level according to Duncan's Multiple Range Test. The two captan and 1.0% Tween control treatments did not differ, and all treatments differed from the water control.

containing ca. 216,000 spores in a shallow, oblique, septic knife cut made into the spring wood vessels 15 cm above the soil level. Disease was recorded at 10 through 444 days after inoculation as percent foliar symptoms estimated visually.

Tree shoots were dissected for observation of vascular browning, and tissues were removed and placed on PDA amended with 200 μg/ml chloramphenicol for detection of *C. ulmi*.

Composite 3-g samples of leaves were diced, extracted in acetone + absolute ethanol (1:1) in a mortar and pestle, filtered, taken to dryness at ambient temperature, and reconstituted with the same solvent system. Aliquots (200 μliter) were pipetted onto 13-mm diam paper assay disks and the disks allowed to dry before being placed on petri plates containing 10 ml PDA amended with 200 μg/ml chloramphenicol. Ten-gram (oven dry weight) composite soil samples were extracted similarly.

For bark and wood analyses, stem sections were removed at random and surface sterilized twice with a 70% ethanol dip followed by flaming. The bark was removed and placed directly onto the agar, while the wood section was split and laid flat-side-down on the agar after which the plates were frozen and thawed quickly; this would hopefully rupture cells, releasing the fungitoxicant for the bioassay.

Benomyl and thiabendazole resides, or derivatives thereof, were detected on the agar by an overspray of spores of *Penicillium expansum* Link or *Verticillium albo-atrum* Reinke & Berth., and captan was detected by *Saccharomyces pastorianus* Hansen (ATCC 21752). The diameter of the inhibition zone indicated the relative quantity of fungitoxicant present.

RESULTS AND DISCUSSION.—No difficulty was experienced in injecting the fungicides, and 6 liters thoroughly saturated the treatment zone. No phytotoxicity was visible in any of the treatments which agrees with the report of Smalley (7).

Foliar symptom development in all trees treated with benomyl (only) was delayed until 25 days after inoculation, while inoculated, untreated control trees began to wilt and exhibited 38% disease after 10 days (Table 1). By 66 days after inoculation, only one of nine trees treated with benomyl alone exhibited symptoms, while three of nine trees treated with benomyl + Tween 80 appeared diseased; the basis for this difference was unknown. Symptoms appeared during the 1971 growing season (444 days after inoculation), a phenomenon observed also by Biehn & Dimond (1) and Smalley (7); six of nine benomyl-treated trees and seven of nine benomyl + Tween 80-treated trees showed disease symptoms. Foliar symptoms, however, occurred at very low levels (4 and 9%, respectively), and no mortality occurred as observed by Smalley (7) at 336 kg/hectare and by Biehn & Dimond (1) at 350 kg/hectare. In all other treatments, every tree had exhibited symptoms within 18 days after inoculation.

Compared to untreated, inoculated check trees, thiabendazole reduced symptoms, but not to a practical extent. The low disease indices, 23 and 28%, observed 444 days after inoculation, were based on recovery and epicormic branching at the basal end of the dead or dying main stem, and do not reflect a tree in vigor and substance comparable to the benomyl-treated or untreated, uninoculated control ones; fresh weights of decapitated tops (Table 2) suggested this. Captan-treated trees were comparable to thiabendazole-treated ones in fresh weight, but exhibited about 30% more symptoms. Although Tween 80 enhanced the movement of these fungicides on soil thin-layer plates (11), the surfactant did not enhance therapeutic action, at least on a practical basis.

Vascular browning, an internal component of the DED syndrome, was present to some extent in at least half of the trees in all treatments (Table 2). The discoloration values for all trees except benomyl-treated ones must be considered conservative, since many trees at the time they were decapitated for analysis were dead, thus making vascular discoloration ratings difficult and uncertain. Likewise, the low frequency of isolation of *C. ulmi*

TABLE 2. Vascular browning in, recovery of *Ceratocystis ulmi* from, and fresh top weight of artificially-inoculated American elm trees 444 days after receiving soil injections of fungicides

Treatment	Percent[1] trees with vascular browning	Percent[1] trees yielding *C. ulmi* in culture	Percent[2] branches yielding *C. ulmi* in culture	Fresh weight (kg) of sacrified, manually-defoliated tree tops
Benomyl	63	13	2	2.27
Benomyl + Tween 80	90	22	4	2.76
Captan	50	0	0	0.59
Captan + Tween 80	100	17	16	0.86
Thiabendazole	77	44	13	0.77
Thiabendazole + Tween 80	75	62	38	1.09
Control, Tween 80, Inoc.	85	43	18	0.86
Control, water, Inoc.	76	16	8	0.59
Control, Uninoc.	0	0	0	2.54

[1] Based upon the number of harvestable trees.
[2] Isolations made from ca. 45 randomly-collected branches from all trees in each treatment.

from all non-benomyl-treated trees probably was due to the invasion by competitive fungi in dead wood that reduced the survival of *C. ulmi*. Biopsy tissues on agar yielded several genera of imperfect fungi. Had isolations been made 66 days after inoculation, a much higher frequency of *C. ulmi* might have been obtained as usual. The low survival of *C. ulmi* in benomyl-treated trees was consistent with low symptom development and high fresh-weight values, and would likely be a factor in recovery in naturally infected trees; the cambium might encase the diseased wood with healthy, uninvaded xylem.

Foliar analyses made two days after treatment revealed the presence of fungitoxicants in trees treated with all three fungicides (Table 3). Since direct chemical characterization of the compounds was not made, this provided only circumstantial evidence that the fungitoxicants present were the soil-injected fungicides or derivatives thereof. Biehn & Dimond (1) detected a fungitoxicant in green shoots and in one-year-old twigs of benomyl-treated elms eight days after soil injection treatment. They noted a positive correlation between quantity of fungitoxicant present in tissues and amount of rainfall. Smalley (7), however, was unable to detect benomyl in trees treated by his trenching method and Hock & Schreiber (3) did not detect benomyl in trees treated by a passive (noninjection) soil placement technique. Hine et al. (2) and Stipes & Oderwald (11) found little or no movement of benomyl and thiabendazole in loam soils. Inasmuch as many landscape elms would likely be growing in loam soil or in those of low sand composition, a passive dry soil placement of fungicides would likely be ineffective as a control measure for DED.

The fungicide soil-injection technique is a latecomer in a series of methods assayed in recent years for the prevention or therapy of DED which include trunk implantations, foliar or bark sprays, soil amendments (dry soil incorporation), drenches, bark poultices to effect sustained uptake, and others. Although all approaches have relative merits as well as disadvantages (10), the soil-injection route, as commonly utilized now in tree feeding, appears to be feasible in certain situations for application of benomyl to elms. Mechanical disruptions or chemical implantations in the trunk or root system of trees are gross injuries which permit invasion of many microorganisms. Many of these bacteria and fungi colonize the tissues in a metabiotic fashion, and death

TABLE 3. Residual fungitoxicants present in American elm tissues and rhizosphere previously soil-injected with fungicides as determined by tissue extraction-paper disk bioassay

Treatment	Zone of inhibition (mm) from various samples[1]				
	Leaves[2]		Bark	Wood	Soil[3]
	old	new			
Benomyl	33	20	+[4]	+	57
Benomyl + 1.0% Tween 80	30	16	+	+	54
Captan	15	16	0	0	25
Captan + 1.0% Tween 80	12	15	0	0	29
Thiabendazole	16	8	+	+	71
Thiabendazole + 1.0% Tween 80	10	5	+	+	71
Control, 1.0% Tween 80	trace[5]	0	0	trace	0
Control, water	trace	0	0	0	0

[1] *Penicillium expansum* was used for benomyl and thiabendazole bioassay and *Saccharomyces pastorianus* for captan.
[2] Sampled 3 months after fungicide application; mean of all samples.
[3] Sampled 17 months after fungicide application; mean of all samples.
[4] + = 5 – 10 mm, a typical fungicide clearing reaction.
[5] trace = 2 or <2 mm, a weak, hazy reaction.

could ensue ultimately from heart-rotting Basidiomycetes (6). Even if colonization and invasion did not occur, one might suspect a reduction in mechanical strength due to repeated, annual disruption and severance of structural fibers and vessels.

Laboratory and greenhouse studies have constituted essential exploratory probes in the initial investigations on fungicidal management of DED. As a sequel to this, the results reported herein have demonstrated the consistency and success of field control of DED by soil injections of benomyl. Further problems need to be resolved before practical use is possible. These include the effective treatment of large landscape-sized specimens, the effective dosage level of benomyl and frequency of application required as well as other considerations. At this point, however, in the fungicidal control history of DED, all feasible methods must be explored and evaluated singly or in combination because topical application, injection and soil treatment methods have merits as well as disadvantages, and each can be unique in every landscape situation.

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