Prevention of Dutch Elm Disease in Large Nursery Elms by Soil Treatment with Benomyl

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ABSTRACT

Soil treatments with benomyl at concentrations of 308 to 382 kg/hectare applied to large nursery American elms prior to bud-break in the spring resulted in significant protection without phytotoxicity following inoculation with *Ceratocystis ulmi*. Thiabendazole [2-(4-Thiazolyl)-benzimidazole] at equivalent concentrations was inactive in these trials. Chemical analysis and bioassays failed to detect benomyl or antibiosis in branches cut from treated trees at the time of inoculation.

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Benomyl has been intensively investigated for control of plant diseases (2, 3, 5), and its systemic fungicidal capabilities in plants treated by soil or foliar application have been well documented (3, 4, 5, 6, 9, 12). It has even been shown to be effective in suppressing or reducing symptoms in the difficult-to-control vascular wilt diseases (1, 2, 5, 6, 8, 9, 12, 14). Several of these reports have suggested that benomyl might be effective in the practical control of Dutch elm disease. Hock & Schreiber (7), however, on the basis of a 1-year study with inoculated nursery elms, concluded that benomyl was ineffective for control of Dutch elm disease when applied to the soil or injected in 50% ethanol into the trunk. I report here the results of benomyl treatments (covering four growing seasons) to control Dutch elm disease in large, inoculated nursery elms.

MATERIALS AND METHODS.—American elms (*Ulmus americana* L.) (6.4 to 10.2 cm diam breast height) used in these investigations were planted in 1958 as whips 2.7 to 3.7 m apart in nursery rows 9.1 m apart at the University of Wisconsin Arlington farms. Except for the benomyl trunk implantation series, trees in a given treatment were utilized in four-tree units covering 14.6 m of nursery row. Such four-tree treatment blocks and controls were randomly scattered throughout a 2.02-hectare planting to provide wide separation of treatments. Trees for trunk implantation were located in a single block of 10 treated elms alternating with 10 untreated controls.

Soil treatments were applied prior to bud-break in the spring as soon as the soil could be worked (2 May 1967; 26 April 1968; 6 May 1969). Chemicals suspended in 18.9 liters of water for each treatment block were dispersed evenly over 134 m^2 (14.6 m x 9.1 m) of soil surface in 15.2-cm deep trenches 15.2 cm apart across the nursery row. The soil type in the treatment area was a well-drained Plano-silt loam. Benomyl for trunk implantations was applied in a thick slurry (2.5 g/tree) in two 2.5-cm diam x 5.1-cm deep holes drilled at a 45 degree angle in the lower trunk.

Elms were inoculated at the anticipated peak of susceptibility (15 June 1967; 6 June 1968; 5-9 June 1969) at two points in different branches in the upper crown in wounds (0.16 cm x 0.63 cm deep) with a conidial suspension of *Ceratocystis ulmi* (Bues.) C. Moreau (ca. 10^6 spores/ml). Conidial suspensions were prepared from a mixture of eight isolates of the pathogen from various Wisconsin localities. Cultures were freshly prepared from infected elm branches which had been previously stored frozen (−15 C) in sterilized, sealed jars. Disease was rated through the growing season following treatment as follows: (i) the number of trees developing limited or systemic wilt; (ii) the degree of crown damage; and (iii) the number of dead or living wilt-free trees.

Because of the limited amounts of fungicide available, the 1967 soil treatments assessed the action of benomyl (382 kg/hectare active material); Thiabendazole [2-(4-Thiazolyl)-benzimidazole] (183 kg/hectare active material); Chemagro 6820 (1, 2-dichloroethyl-*p*-chlorophenyl sulfone) at 58 kg/hectare; Chemagro 6698 (2,4-dichloro-6-nitrophenylphenoxy acetate) at 67 kg/hectare; and Fintrol-5 (antimycin A) at 24 kg/hectare; in single four-tree treatment blocks, at the manufacturer's suggested dose. In 1968, the benzimidazole fungicides were contrasted at more comparable doses (benomyl at 308 kg/hectare, Thiabendazole at 412 kg/hectare) utilizing five four-tree blocks/compound or control. Sufficient benomyl was available in 1969 to complete a dosage-response experiment (0, 1, 11, 56, 112, 224 kg/hectare). Five four-tree blocks were treated/dose. Branch samples for bioassay and chemical determination of benomyl concentrations were cut from one tree in each block of the dose-response trial at the time of inoculation and stored frozen (−15 C) in sealed polyethylene bags. Bioassay procedures were similar to those described by Hock et al. (9), and chemical extractions and analysis for benomyl followed the colorimetric procedures of Pease & Gardiner (10). Untreated branch samples spiked with

TABLE 1. Occurrence of symptoms of Dutch elm disease in inoculated American elms treated with systemic fungicides by soil application and trunk implantation

Treatment	Trees	Dates of symptom observations: 1 Sept. 1967, Trees with systemic wilt	1 Sept. 1967, Avg crown damage	28 Aug. 1968, Trees dead	28 Aug. 1968, Avg crown damage
	no.	*no.*	*%*	*no.*	*%*
Soil application[a]					
Control (no treatment)	24	15	42	10	45
Antimycin A (23 kg/hectare)	4	2	50	3	75
Chemagro 6698[c] (67 kg/hectare)	4	3	75	3	75
Chemagro 6820[d] (58 kg/hectare)	4	2	48	2	50
Thiabendazole[e] (183 kg/hectare)	4	2	31	2	50
Benomyl (382 kg/hectare)	4	0	2	0	0
Trunk implantation[b]					
Control (no treatment)	10	7	54	7	70
Benomyl (2.5 g/tree)	10	4	40	6	60

[a]Treated 2-5 May 1967; inoculated 15 June 1967.
[b]Treated 16 May 1967; inoculated 15 June 1967.
[c]2,4-dichloro-6-nitrophenylphenoxy acetate.
[d]1,2-dichloroethyl-*p*-chlorophenyl sulfone.
[e]2-(4-Thiazolyl)-benzimidazole.

analytical-grade benomyl served as controls for the analysis procedures.

RESULTS AND DISCUSSION.—None of the elms treated in 1967 soil applications of benomyl (382 kg/hectare) developed systemic wilt, and all trees remained healthy during the season following treatment (Table 1). By the end of 1968, the inoculated untreated elms or elms treated with the soil applications of antimycin A, Chemagro 6698, Chemagro 6820, and Thiabendazole had developed 45% or more crown damage, and almost half the trees were dead. The Thiabendazole-treated elms had a slightly reduced average crown damage as compared to controls during 1967, but these differences disappeared the following year. Benomyl trunk implantations slightly reduced the numbers of trees developing systemic wilt, but these differences also disappeared. General disease levels in the 1967 treatments were somewhat low (45% crown damage in controls) indicating that the highest level of Dutch elm disease susceptibility had passed prior to inoculation. In spite of this, the benomyl "control effect" indicated above was impressive.

The more extensive soil treatments with benomyl and Thiabendazole in 1968 produced results similar to those obtained in 1967 (Table 2). In this series, the general level of disease was quite high (79% crown damage in controls), and elms were probably near their peak of susceptibility. The level of control obtained from the benomyl treatments in the season of treatment, however, declined somewhat the next year. Disease development in the Thiabendazole treatments was extensive, and did not differ from the untreated controls.

The dose-response benomyl soil treatments in 1969 largely confirmed the results of the earlier trials. No reduction in disease was evident until benomyl dosages reached 224 kg/hectare or higher (Table 3). Reduced amounts of crown damage as compared to controls were recorded at 224 kg/hectare during 1969, but these effects disappeared in 1970. At 336 kg/hectare, the control effects slightly exceeded those

TABLE 2. Occurrence of symptons of Dutch elm disease in inoculated American elms treated with substituted benzimidazole fungicides by soil application[a]

Treatment	Trees	Dates of symptom observations: 28 Aug. 1968, Trees with systemic wilt	28 Aug. 1968, Avg crown damage	8 Oct. 1969, Trees dead	8 Oct. 1969, Avg crown damage
	no.	*no.*	*%*	*no.*	*%*
Control (no treatment)	20	16	72	15	79
Thiabendazole[b](412 kg/hectare)	20	17	75	16	80
Benomyl (308 kg/hectare)	20	10	31	9	50

[a]Treated 26 April 1968; inoculated 6 June 1968.
[b]2-(4-Thiazolyl)-benzimidazole.

TABLE 3. Occurrence of symptoms of Dutch elm disease in inoculated American elms treated by soil application with different concentrations of benomyl

		Dates of symptom observations			
		8 Oct. 1969		6 July 1970	
Treatment[a]	Trees	Trees with systemic wilt	Avg crown damage	Trees dead	Avg crown damage
kg/hectare	*no.*	*no.*	*%*	*no.*	*%*
0	20	13	54	13	65
1	20	13	51	11	59
11	20	16	65	15	80
56	20	16	56	15	75
112	20	15	63	15	75
224	20	13	40	11	60
336	20	8	21	6	33

[a]Treated 6-9 May 1969; inoculated 6 June 1969.

obtained at 308 kg/hectare the previous year, and these levels did not change during the 1970 season.

No phytotoxicity was evident from any of the soil treatments in these studies with any of the chemicals tested. Benomyl treatments at 224 kg/hectare or higher in inoculated elms consistently resulted in more surviving elms and lower levels of crown damage than untreated controls. Some trees, however, died in most benomyl treatment blocks except at the highest dose (382 kg/hectare) in which all trees survived. No benomyl could be detected in any of the 35 branch samples cut from trees at the time of inoculation in the 1969 dose-response trial. This proved to be the case for both the bioassay technique for analysis (9, 11) and for the complex extraction and colorimetric chemical analysis (10). From this, and from the fact that treated trees died occasionally, it could be concluded that benomyl concentrations in the elm branches were quite low at the time of inoculation even at the highest levels of soil application (336 kg/hectare), and probably never exceeded 0.1 mg/kg, the reported sensitivity of the chemical analysis (10). In general, these results were similar to those of Biehn & Dimond (1), but contrary to those reported by Hock & Schreiber (7). Biehn & Dimond (1) applied benomyl at 291 and 454 kg/hectare (260 and 405 lb./acre), and Hock & Schreiber (7) at 434 kg/hectare (90 g/26 ft^2). Possibly Hock & Schreiber's mid-June treatments were applied too late to obtain adequate distribution of the fungicide in the tree prior to *C. ulmi* infection.

None of the other soil fungicides used in these trials resulted in control of Dutch elm disease. Even Thiabendazole, a compound chemically related to benomyl, applied at 412 kg/hectare failed to protect the inoculated elms from the fungus. However, at this concentration it proved to be a highly effective herbicide against *Taraxacum officinale* Weber. Benomyl trunk implantations also failed to control the disease (Table 1), as did injections reported by Hock & Schreiber (7).

The degree of protection achieved by benomyl soil treatments, although consistent in three seasons of treatment, was not as high as might have been hoped for in a chemical to be used in the practical control of Dutch elm disease. For example, in trials with similar nursery elms, much higher levels of protection were obtained with injections of the plant-growth regulator, sodium 2,3,6-trichlorophenyl acetate (13). The high rate of benomyl soil treatment necessary to produce significant control would seem to limit the potential usefulness of this type of treatment in Dutch elm disease control to certain high-value elms. In terms of scientific significance, however, benomyl might constitute the first systemic fungicide to be recognized with capability of producing significant practical protection in large, field-grown American elms inoculated with *C. ulmi*. Its actual mechanism of action needs to be clarified, and a more effective means of introducing the chemical into elm trees needs to be discovered.

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