## Pressure Injection of Benomyl and Methyl-2-benzimidazolecarbamate hydrochloride for Control of Dutch Elm Disease

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## ABSTRACT

The effectiveness of benomyl (50% WP formulation) against Dutch elm disease was tested by pressure-injecting aqueous suspensions into trunks of large urban trees and small plantation trees. Trunk injection of benomyl was equally as effective as a spray program with methoxychlor for protecting large trees, but was totally ineffective on artificially inoculated small trees. Methyl-2-

benzimidazolecarbamate hydrochloride very effectively protected the smaller trees against the disease. Bioassays showed that benomyl suspensions moved poorly through elm stems, and that only ca. 10% of the fungitoxicity could be detected after the suspension had passed through 20 cm of stem.

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Benomyl fungicide [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] has been shown to be effective as a protectant against Dutch elm disease when applied as a spray (7), by trunk injection (12), or by soil application (2,3). Methyl-2-benzimidazolecarbamate (MBC), a fungitoxicant derived from benomyl (10), has also proven to be effective in preventing Dutch elm disease when applied by trunk injection (5) or root injection (9).

Smalley et al. (12) reported that benomyl, injected by Mauget cups (J. J. Mauget Co., Burbank, Calif.), can

cure diseased elms. This curative property has also been reported for MBC (5).

The reported effectiveness of trunk injections of benomyl in both protecting against and curing Dutch elm disease is puzzling, because the material injected into the tree'is a 50% wettable powder formulation of a fungicide with very low water solubility (1), and thus, the suspension entering the tree would not be expected to move readily in the tree's vascular system.

The objectives of this study were: (i) to compare

benomyl trunk injection of large elms by commercial arborists with the commonly-used methoxychlor protectant sprays; (ii) using small plantation elms, to test the effectiveness of benomyl trunk injections as a protectant and eradicant, and to compare its efficacy as a protectant with MBC·HCl trunk injections; and (iii), to test the ability of benomyl and MBC·HCl to move through elm stems.

MATERIALS AND METHODS.—Large urban trees. - American elm trees (Ulmus americana L.) located near streets or in private gardens and varying in size from 25 to 125 cm in diam at breast height (dbh), were treated either with a spray schedule of methoxychlor (2,2-bispmethoxyphenyl-1,1,1-trichloroethane) or by trunk injection of benomyl fungicide (Benlate 50 WP) during May and June, 1973 in cooperation with a local arborist (Walgren Tree Experts, Inc., West Hartford, Conn.). The injections were made with Model 102-C pressure injectors from the Elm Research Institute (Harrisville, New Hampshire) at a pressure of 2 bars. Benomyl was used at a concn of 2 g active material per liter, and at least 31 ml was injected per 2.54 cm circumference at breast height (usually between two and three times this volume was injected into each tree). Benomyl was injected once during either May or June. Trees treated with methoxychlor received a prefoliar spray of the insecticide in early May and a second spray at the end of May. These sprays were applied with mist blowers (John Bean, Model 300) using 12.5% methoxychlor for the prefoliar spray and 6.2% for the second spray. The treated trees were distributed throughout an area of approximately 20 square km.

In June 1973, after all treatments had been completed, the trees were visually inspected for symptoms of Dutch elm disease and stem sections were cut from the crown of the trees for isolation of *Ceratocystis ulmi* (Buisman) C. Moreau where there was a question concerning the cause of wilting. On the basis of this survey, it was concluded that the two groups of trees, the benomyl-injected (130)

trees) and the methoxychlor-sprayed (88 trees), were comparable, each having approximately the same percentage of trees with symptoms of Dutch elm disease (18% and 20%). A second survey was made in September 1973, evaluating those trees which had been found to be disease-free in the June survey.

Bioassays were done on small branches from the crown of trunk-injected elm trees. Disks were cut from the branches and placed on potato-dextrose agar (PDA) plates seeded with *Penicillium* sp. spores. Inhibition of fungal growth on the agar surrounding the stem disk was used as the criterion for the presence of a fungitoxic compound.

Plantation trees.—American elm trees ranging in size from 7.5 cm to 15 cm dbh were selected at random from an experimental planting at Lockwood Farm of the Connecticut Agricultural Experiment Station located in Hamden, Connecticut. The Elm Research Institute pressure injector was used to apply the treatments at a pressure of 2 bars through five 9-mm diam holes drilled in the base of the tree. Benomyl suspension was applied at the recommended rate of 472 ml per 24.4 cm basal circumference using 2.0 g active material per liter of water (11). MBC·HCl was used at approximately the highest concn (4.5 g per liter of 0.05 N HCl) not showing phytotoxicity as reported by Gregory et al (5). Both water and 0.05 N HCl were injected separately as checks. To inoculate the trees, 0.1 ml of water containing about 10<sup>6</sup> conidia was placed in a 2- to 4-mm deep cut in the bark of a small primary branch. The cut was made within 15 cm of the trunk. MBC·HCl was prepared according to the method of McWain and Gregory (10).

Pressure chamber experiments.—A pressure chamber (13) was used to evaluate the flow of benomyl suspensions and MBC·HCl through elm stems using the same concust that were injected into the trees. Elm stems 3-5 mm in diam and 20 cm long were placed in the fluids to be tested with all but the top 5-cm of the stem immersed in the fluid.

TABLE 1. Results of pressure injection of benomyl (2 g active ingredient/liter) and MBC·HCl (4.5 g/liter) into trunks of plantation elms

Time of inoculation	Material injected		Crown wilting <sup>a,b</sup> (%)	No. of trees wilting <sup>a,b</sup> (%)	$P^c$
1 wk after injection					
AND CONTRACTOR	Benomyl 50 WP		30	80	
	$H_2O$		56	100	< 0.200
	MBC·HCl		8	20	33.003
	HCl (0.05N)		49	100	< 0.005
Immediately after injection	***				
	Benomyl 50 WP		41	80	
	$H_2O$		57	100	< 0.400
1 wk before injection				2	301.100
100 m 200 m	Benomyl 50 WP	*	64	100	
	H <sub>2</sub> O		53	80	< 0.500

Average of 10 trees.

bSymptoms 6 wk after treatment.

Data analysis by t-statistics for significant differences in means of unpaired observations.

The cylinder containing the fluid and stem were set in the pressure chamber. Polyethylene capillary tubing was inserted through an airtight seal to the top of the upper cut-end of the elm stem. The fluids passing through the elm stem under pressure (2 bars) flowed from the pressure chamber through this tubing and was collected in a graduated cylinder. Stem conductance was determined by measuring the time required for each 0.5 ml of fluid to pass through the stem.

RESULTS.—Large urban trees.—The results of the initial survey showed that 107 of the benomyl-treated trees, and 70 of the methoxychlor-treated trees, were healthy in June 1973. By September, symptoms had appeared on 10 (9.4%) of the previously healthy benomyl-treated trees, and three (4.3%) of the previously healthy methoxychlor-treated trees. Using a contingency test, it was found that there were no significant differences between these results.

Stem tissue from six of the benomyl-treated trees was assayed in June for the presence of fungitoxicity in the crown of the tree. Five of the trees assayed contained some fungitoxic compound in parts of the crown. However, not all the stem disks from any one tree showed fungitoxicity, indicating that the fungicide was not well-distributed throughout the tree.

Plantation elms.—Trunk injections of benomyl into the small elms did not control Dutch elm disease when applied either as an eradicant or as a protectant (Table 1). Samples were taken from all parts of the crown of some treated trees for detecting fungitoxicity. The bioassay with Penicillium could not detect benomyl in any of these samples.

MBC·HCl effectively protected the trees against Dutch elm disease (Table 1).

Pressure chamber.—Comparing the conductance of water through the elm stems with the conductance of benomyl suspensions and MBC·HCl solutions showed the conductance of benomyl was significantly lower than water and MBC·HCl, and that the conductance of benomyl decreased in proportion to the amount forced into the stem (Fig. 1). This is consistent with the observation that it takes much longer to inject benomyl into trees than it does to inject water or MBC·HCl. The fluid collected after the benomyl was forced through the 20-cm-long stems was clear, indicating that the suspended particles had been filtered out by the stem. When the solution that had passed through the stem was bioassayed using absorbent paper disks (Schleicher & Schuell, Inc.) on PDA plates seeded with Penicillium sp. spores, it was found that this solution averaged only 11% of the fungitoxicity possessed by the original benomyl suspension. However, when MBC·HCl was passed through stems, none of the fungitoxicity was lost.

DISCUSSION.—Our results indicate that application of benomyl by trunk injection is probably not the best method for controlling Dutch elm disease. The method was totally ineffective in acting as either a protectant or cure for the disease in small plantation elms. The inability of the method to control the disease in the small, artificially inoculated trees may have been due to the inoculation method which is particularly favorable for introducing the fungus. The location of the wound was close to the trunk and thus allowed rapid spread of the

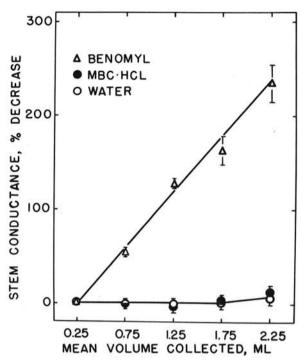


Fig. 1. Conductance of benomyl (4 g Benlate 50 WP/liter), MBC·HCl (4.5 g/liter), and water through elm stems 3-5 mm in diam and 20 cm in length. Results expressed as percent decrease in conductance (cm³/bar sec) of each 0.5 ml of fluid passed through the stems vs. the mean of each 0.5 ml volume. The vertical lines represent the range of two times the standard error.

fungus throughout the tree, and the number of spores introduced was much greater than the number ordinarily carried by the beetle vector. Even so, this unnaturally large inoculum could still be controlled by MBC·HCl (Table 1).

Since, in our tests, benomyl was no more effective in preventing natural infection in large trees than was methoxychlor, preference must be given to methoxychlor as the better control method. Methoxychlor is cheaper to apply, and does not cause wounds which could lead to the introduction of decay organisms or unsightly wet wood conditions. The latter condition has been observed emanating from the wounds caused by the injection apparatus in a few trees.

Unfortunately, because of the value of large elms in our area, we did not have untreated control trees; thus, we do not know the natural level of infection to compare with the level of infection in the treated trees. It is possible that neither of the treatments were effective in preventing the disease. We would certainly question the effectiveness of any method that allows 5-10% of the healthy trees to become infected in any one year. Such a level of protection may be reasonable for an annual crop but is unacceptable for trees having a life expectancy of over 100 yr.

The poor performance of benomyl in controlling the disease may be explained by its behavior when passing through elm stems. Our experiments show that benomyl suspensions flow very poorly through elm stems and that

the more going into a stem, the slower the flow will be. When benomyl is injected into a tree, most of the suspended materials are probably filtered out and remain close to the point of injection. The fact that approximately 90% of the fungitoxicity of the benomyl suspension was lost after passing through only 20 cm of small elm stems under pressure, is evidence that this is occurring. This is in contrast to MBC·HCl solution which moved through the elm stems at the same rate as water and lost none of its fungitoxicity after passing through 20cm-long stems. The difficulty we and others (4, 8, 12) have had in obtaining positive bioassays for the presence of fungitoxicity in the crown of benomyl-treated trees as compared with MBC·HCl-treated trees (12) is further evidence that benomyl moves poorly through the vascular system of elms as compared with MBC·HCl.

Our results indicate that a water-soluble fungicide such as MBC·HCl is preferable to an insoluble one such as benomyl. In our experiments where the two fungicides were compared, the MBC·HCl was much superior to benomyl in controlling the disease. The difference in the effectiveness of the two fungicides is certainly not due to a difference in their fungitoxicity (6) but undoubtedly is a function of their water-solubility. MBC·HCl should be further tested as a treatment for Dutch elm disease.

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