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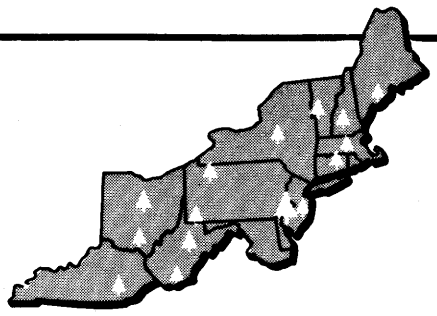

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PRESSURE INJECTION OF METHYL 2-BENZIMIDAZOLE CARBAMATE HYDROCHLORIDE SOLUTION AS A CONTROL FOR DUTCH ELM DISEASE

Abstract. A preliminary evaluation of the effectiveness of injecting methyl 2-benzimidazole carbamate hydrochloride solution into elms for prevention or cure of Dutch elm disease is reported. Symptom development was diminished or prevented in elms injected with fungicide before inoculation. Symptom development was arrested in all crown-inoculated diseased trees injected with the high and medium concentration of the fungicide and in bole-inoculated diseased trees injected with the high concentration of the fungicide when not more than about 50 percent of the crown was symptomatic.

Benomyl (1-(butylcarbamoyl)-2-benzimidazole carbamic acid, methyl ester) is a broad-range systemic fungicide that has been widely investigated during the last few years as a possible control agent for various plant diseases.

Benomyl has been used experimentally in attempts to control Dutch elm disease (DED). Various workers have used soil drenches (1,5,11,12) and spray application to the crown (4,11) in an effort to protect elms from DED, with results ranging from none to apparently good protection.

Soil drenches and fungicide sprays are objectionable in several respects. Application by soil drench results in variable uptake of benomyl, dependent on soil type and

probably on soil moisture. This method requires large amounts of fungicide and allows little control over its subsequent fate. We have little understanding of its effect on soil microbiology. Similarly, spray application requires relatively large amounts of the chemical, much of which eventually is deposited in the soil. Drift of spray onto or into houses, cars, etc., is also undesirable.

On the other hand, pressurized injection of fungicide directly into the xylem of the tree seems to offer many advantages over the other two methods. Solubilized benomyl injected into the xylem of elms, oaks, and maples becomes distributed in the branches and twigs (3). With injection the fungicide is confined within the tree, so the environmental contamination hazard appears to be minimal. The time between treatment and establishment of an effective dosage at desired sites is appreciable for soil applications but short for injections.

Benomyl hydrolyzes to methyl 2-benzimidazolecarbamate (MBC), butylamine, and carbon dioxide. MBC is considered to be the active fungicidal material in plants that receive applications of benomyl (10). Buchenauer and Erwin (2) applied a spray containing MBC to cotton plants for the control of Verticillium wilt. Certain types of benzimidazolecarbamates were introduced as fungicides in patents by Klopping (7). Littler et al. (8) patented the use of MBC and some of its fungitoxic salts as foliar fungicides. McWain and Gregory (9) devised a laboratory-scale procedure for conversion of benomyl to water-soluble hydrochloride salt of MBC (MBC·HCl) that was used in these tests.

This is a report of the preliminary results of studies to determine the value of pressure injection of the MBC·HCl solution into the xylem of American elms for prevention and therapy of Dutch elm disease.

Methods

The methods were similar for the two studies, benomyl prophylaxis and benomyl therapy of Dutch elm disease in Ulmus americana, American elm.

Study trees were divided into four size classes. Each treatment of the two studies was randomly assigned to one tree in each size class so each treatment was repeated four times. Four replications of each treatment were in nursery trees of 5 to 11 inches d.b.h.

Prophylactic Study

Trees were injected using the pressure injection apparatus and method described by Jones and Gregory (6). Injected solutions were 0.00, 0.67, 4.0, and 24 g./l. MBC·HCl. Injected volumes were approximately proportional to the d.b.h. of the trees (about 325 ml./inch d.b.h.). The number of injection sites per tree was two for 5- to 9-inch trees or three for 10- to 13-inch trees. The injection sites were evenly spaced around the tree trunk, 1 to 3 feet above the ground. Injection pressure was 40 p.s.i. Injection wounds were covered immediately after injection with tree wound dressing.

One week after injection, the trees were inoculated in one of two ways: through 1/2-inch wood chisel cuts into the xylem at 4-inch intervals around the bole of the tree at breast height, or through one 1/2-inch wood chisel cut into the xylem of a branch located about midway between the top and bottom of the crown and where that branch was about 1 inch in diameter. Twenty-five million conidia of an aqueous suspension of a mixture of five isolates of *Ceratocystis ulmi*, were applied to each chisel cut. These particularly severe inoculation procedures were used to test the value of the MBC·HCl injection treatments rigorously.

Treatment checks consisted of trees that were treated but not inoculated and inoculation checks consisted of trees that were inoculated but not injected.

Detection of fungitoxicant in the branches of treated trees was attempted 2 weeks after injection and then at monthly intervals throughout the remainder of the growing season. Branch tip samples were collected from the north, east, south, and west sides of each tree at about midcrown height. Samples were refrigerated until processed in the laboratory. A 1/4-inch-long section from each sample was placed in the center of 10-cm.-diameter petri plates containing 30 ml. of potato dextrose agar, in which 450,000 conidia of *Penicillium* sp. were dispersed. Zones of *Penicillium* growth inhibition were measured after 48 hours' incubation at room temperature.

Observations of foliar symptoms were recorded at approximately weekly intervals throughout the growing season. Near the end of the season injection sites were examined for injury, and the extent of the dead tissue, if any, was recorded.

Therapeutic Study

Trees were inoculated as described for the prophylactic study. Injections, as previously described, were made at 70 p.s.i. with either 4 or 24 g./l. of MBC·HCl when trace, 25 percent, or 50 percent crown symptoms were apparent.

Inoculation checks consisted of trees that were inoculated but not treated. Treatment checks in the prophylactic study were considered applicable to this study, as well.

C. ulmi infection in trees of both studies was confirmed by pathogen isolation from branch samples.

Results and Discussion

Bole-inoculated trees of the prophylactic study showed less symptom development with greater concentrations of injected MBC·HCl (table 1). Symptom development was limited to an average of 8 percent of the crown in trees treated with the highest concentration. This is especially impressive in view of the extreme severity of the inoculation. Furthermore, only 50 percent of the trees treated with the medium and high concentrations developed any symptoms.

The crown-inoculated trees showed markedly less symptom development at all concentrations of the fungicide, and, at the highest concentration, no symptom development at all.

In the bole-inoculated trees of the therapeutic study there was little difference in symptom development between noninjected check trees and those injected with 4 g./l. of MBC·HCl at trace, 25, and 50 percent crown symptom development (table 2). There were markedly fewer symptoms in those injected with 24 g./l. at trace and possibly at 25 percent symptom development. It is important to recognize that in bole-inoculated trees, very rapid disease development occurred almost at the same time throughout the crown. This, in practice, meant that disease development was actually much more advanced at the time of treatment than is indicated by the assigned value of trace, 25, or 50 percent symptoms (table 2).

Symptom development was curtailed and some remission of symptoms occurred in crown-inoculated trees injected with either 4 or 24 g./l. of fungicide at trace, 25, and 50 percent symptoms (table 2). Trees injected at 25 percent symptom development with 4 g./l. MBC·HCl are possible exceptions to this conclusion.

Table 1.--Prophylactic study: development of DED crown symptoms at the end of the 1972 growing season in inoculated trees previously injected with MBC·HCl solution

Treatment	Average percentage of crown symptomatic[a/]			Percentage of trees symptomatic[a/]	
	Noninoculated checks	Bole inoculated	Crown inoculated	Bole inoculated	Crown inoculated
No injection	0	100	50	100	100
Water injection	0	88	73	100	100
Benomyl injection					
0.67 g./l. conc.	0	63	6	100	50
4.0 g./l. conc.	0	24	28	50	50
24.0 g./l. conc.	0	8	0	50	0

[a/] Average of four replicates.

Table 2.--Therapeutic study: average[a/] percentage of DED leaf symptoms at time of injection and at end of 1972 growing season in inoculated trees injected with MBC·HCl solution

MBC·HCl concentration	Inoculation site	Percent symptoms-- At time of injection	Percent symptoms-- At end of growing season
Trees designated to be injected at trace symptoms[b/]			
4 g./l.	Bole	60	85
4 g./l.	Crown	3	5
24 g./l.	Bole	53	55
24 g./l.	Crown	3	14
Trees designated to be injected at 25% symptoms[b/]			
4 g./l.	Bole	70	78
4 g./l.	Crown	37	53
24 g./l.	Bole	50	68
24 g./l.	Crown	26	18
Trees designated to be injected at 50% symptoms[b/]			
4 g./l.	Bole	55	88
4 g./l.	Crown	57	53
24 g./l.	Bole	73	96
24 g./l.	Crown	58	58
Controls			
No injection	Bole		85
	Crown		80

[a/] Average of four replicates.

[b/] Trees frequently developed symptoms rapidly and consequently could not be injected at the designated percent symptoms. This is particularly true for the bole-inoculated trees.

In both studies, the time required for solution injection varied with the health of the tree and with the volume and concentration of the solution. However, most of the trees required less than 30 minutes.

No apparent injury to the foliage resulted from injection of any of the MBC·HCl solutions. Injury to the tissue for variable but usually short distances above and sometimes below the injection sites was observed at about 70 percent of the injection sites on trees treated with 24 g./l. of MBC·HCl. The injection sites on trees treated with 4 g./l. MBC·HCl or less showed little or no injury. Larger trees had less injury than the smaller ones. Active callus tissue was present around most injection sites and some were nearly healed over by the end of the season. Most of the trees injected with 4 and 24 g./l. MBC·HCl yielded some positive bioassays. However, few of the trees that received 0.67 g./l. MBC·HCl had positive bioassays.

In conclusion, it appears that injection of 24 g./l. MBC·HCl provided nearly complete protection from Dutch elm disease in American elms under conditions of this study, and either the high or medium concentration generally checked symptom progression in infected trees. However, it should be emphasized that these are preliminary results and it remains for observations in the spring and summer of 1973 to determine the permanence of the disease control.

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