Benomyl for Practical Control of Dutch Elm Disease

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ABSTRACT

Remission of naturally or artificially induced Dutch elm disease in its early stages was achieved in a high percentage of elms treated by trunk or soil injection with benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate. Early, fast-developing infections, such as occur with root graft transmissions or recurrent disease were not controlled by such treatments except in cases of recurrent disease where treatment was combined with removal of the infected branches. Mass protection was achieved in municipal situations by spring application of benomyl either by trunk injection or by mist-blown foliar sprays. In such treatments, the incidence of new infection in untreated trees was 3 to 4 times greater than in the treated trees. Analysis and bioassay of greenhouse-grown elms sprayed with benomyl formulations suggested that systemic uptake of benomyl was accomplished in small branches through the lenticels. Improved solubilization of the benomyl without phytotoxicity was achieved using acetone-HCl solutions and in suspension with Tween 80, polyoxyethylene sorbitan monoleate, and Tergitol NPX, polyglycol ethers. Lactic acid solubilization yielded high antifungal activity, but solutions were extremely phytotoxic.

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The action of benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, in prevention of Dutch elm disease by soil applications has been clearly established (4, 22). The high rates necessary to achieve significant protection of healthy elms (380 to 450 kg/hectare), however, would seem to limit the potential usefulness of this type of treatment to certain high-value elms in any practical Dutch elm disease control program.

Foliar sprays would not appear to be a very promising alternate method of application, since past investigations with benomyl indicate that the direction of systemic movement is usually upward or distally in the leaves (1, 17, 18, 19, 20). The functioning xylem at the twig crotch infection court of *Scolytus multistriatus* Marsh. transmissions would thus appear to be an unlikely place for benomyl accumulation. However, Buchenauer & Erwin (5) have reported both therapeutic and protective action of benomyl foliar sprays in the Verticillium wilt disease of cotton (*Verticillium albo-atrum* Reinke & Berth.), if benomyl is first converted to the water soluble methyl-benzimidazolecarbamate-HCl. Busch & Hall (6) reported similar prevention of Verticillium wilt of chrysanthemum by foliar applications of benomyl. They made use of Tween 20 (polyoxyethylene sorbitan monolaurate) to increase the water solubility of benomyl. Recently Hart (9) reported success in protecting elms from Dutch elm disease by repeated mist-blown foliar sprays of benomyl with Bio-film (alkylarylpolyethoxyethanol).

High pressure trunk injection of solubilized benomyl has been suggested as a practical approach to the introduction of protectant or therapeutic doses of benomyl into elms (8, 11, 13, 14). The effectiveness of these techniques in foliated elms naturally or artificially inoculated with *Ceratocystis ulmi* (Buism.) C. Moreau apparently remains to be established.

We report here the results of laboratory, greenhouse, and municipal field investigations to develop improved techniques for application and bioassay of benomyl when applied to elms to prevent or cure Dutch elm disease.

MATERIALS AND METHODS.—*Plant materials.*—Healthy American elms, *Ulmus americana* L. for greenhouse foliar spray trials were grown in 10-cm diam pots in Jiffy potting mix (Jiffy Products of America, Chicago, Illinois) at 19 C night, 21 C day, temperatures under a 16-hr day provided by incandescent illumination at 10,760 lx (1,000 ft-c) at 100 cm from the source. Buds of 1-year-old seedling stock were pruned to produce plants with a single stem 97 cm (average) tall at the time of treatment. Elm seedlings transplanted from moist vermiculite in the second-true-leaf stage into 10-cm diam pots in U. C. mix (3) were grown under the same greenhouse conditions, and sprayed when the plants had reached 60 cm (average) in height.

The healthy municipal elms for protective sprays were street-border trees located in the city of Milwaukee. The elms for protective and therapeutic trunk and soil injections, or for bioassay, were situated in the Wisconsin villages of River Hills,

Bayside, Whitefish Bay, Shorewood, Fox Point, in our elm nursery on the University Arlington Farms, and in the cities of Madison and Waukesha. The trees in Fox Point, Bayside, Whitefish Bay, Shorewood, and Waukesha were mainly street-border trees; whereas those in River Hills were located on estate lawns or on the Milwaukee Country Club grounds. Early-stage current season infections for therapeutic treatment were located initially by ground survey, and in later studies by weekly visual surveys in a low-flying helicopter.

Improved formulations.—Methods for solubilization of benomyl as suggested by various authors (5, 6, 14, 23) were tested in vitro for possible application in greenhouse and field investigations. Benomyl stock solutions or suspensions were prepared at concentrations of 50 g/liter of distilled water or solvent. All benomyl concentrations are expressed as grams active ingredient. The stock solutions in 85% lactic acid were warmed at 70 C for 1-2 hr to aid solubilization. Methods for preparing methyl-benzimidazolecarbamate-HCl (MBC-HCl) suggested by Buchenauer & Erwin (5) were modified to prepare a more concentrated stock solution. Thus, 25 g active benomyl was dissolved at 24 C in 500 ml acetone, warmed in a water bath to 70 C over a 30-min period, and mixed with 400 ml of hot (70 C) 0.35 N HCl. This resulted in instant conversion of the milky suspension to a clear amber-brown liquid. After cooling, the final volume was adjusted to 1 liter with 0.35 N HCl.

Dilutions were prepared to give benomyl concentrations from 0.5 to 25 g active ingredient/liter. Surfactant concentrations [Tween 20, Tween 80 (polyoxyethylene sorbitan monooleate), Tergitol NPX (polyglycol ethers), Regulaid (Polyoxyethylenepropyl-propoxypropanol + alkyl-2-ethoxyethanol), Plyac (Emulsifiable A-C polyethylene + octyl polyoxypolyethoxyethanol) and Biofilm] at each benomyl level were prepared at 1, 2.5, 5.0, and 10 ml product/liter of benomyl suspension.

Fungicidal activity of the 72-hr standing solutions and freshly agitated suspensions was determined in vitro by the filter paper disk method (12.7 mm disks, Schleicher & Schuell, Inc., Keene, New York) (24). Inhibition zones on potato-dextrose agar (PDA) seeded with conidia of *C. ulmi* were measured after 96 hr incubation at 24 C. Phytotoxicity was determined by dipping turgid, freshly cut, 10- to 14-mm wide elm leaf strips in each solution or suspension (three strips/preparation). Leaves for this purpose were harvested from a single vigorous American elm growing on the University Campus. Phytotoxicity after 48 hr incubation in the light (16 hr day) in petri dishes over moist filter paper was scored as: 1 = nonphytotoxic, 2 = slight marginal water soaking or browning, 3 = moderate marginal browning with necrotic spots, 4 = severe browning, 5 = complete necrosis. Detached leaf phytotoxicity scores were nearly identical to those obtained using similar formulations sprayed on foliage of greenhouse-grown elms, and also correlated well with internal xylem discoloration produced after trunk injection of similar solutions.

Artificial inoculations made in River Hills and Fox Point were accomplished as previously reported (21) utilizing a truck-mounted hydraulic lift to reach the upper crown branches. Natural inoculations were accomplished by resident elm bark beetle populations (mainly *S. multistriatus*).

Bioassays.—Previously described bioassay procedures (12) and direct chemical analyses (16) were not adequate to detect xylem antifungal activity at apparent protective benomyl doses (21). Although inhibition zones rarely formed, the fungus frequently failed to grow on xylem segments from benomyl-treated branches on *C. ulmi*-seeded agar plates even after 4-week incubations. A modified bioassay was developed making use of this fact.

Branch segments to be assayed were stored with leaves removed in sealed polyethylene bags and, where necessary, held at 5 C until processed (usually not more than 2 weeks). For the assay, branches were cut into 6-cm lengths, the bark removed aseptically, and the nonsterile ends of each branch trimmed with a sterile clipper. The xylem segments were transferred to *C. ulmi*-seeded PDA plates, and incubated for 2 weeks at 24 C under a 16-hr day (Fig. 1). Results of replicated treatments were scored as follows: 1 = no growth of the fungus, 2 = slow developing fungal growth, 3 = fair mycelial growth with limited coremial development, 4 = good mycelial growth with fair coremial development, 5 = heavy mycelial growth with intense coremial production. When high concentrations of antifungal compounds were present, inhibition zones also developed (Fig. 1).

Whole treated trees were bioassayed by incubating either 4-mm diam increment borings or 0.5-cm-thick trunk and branch disks, cut with a band saw from various parts of the tree, for 14 days on *C. ulmi*-seeded plates (Fig. 2). Coremial development occurred only in areas without the antifungal activity. This technique, in combination with the branch bioassay, was used to map the distribution of activity throughout whole treated elm trees (Fig. 3).

Foliar applications.—In the greenhouse, paired elms were sprayed to drip with benomyl at five different concentrations (0.25 to 18.1 g active principle/liter). Benomyl was applied either suspended in water or mixed with NuFilm 17 (*di*-1-*p*-menthene-terpene polymer), 10 ml/liter; Tergitol NPX, 1 ml/liter; Tween 20, 1 ml/liter; Tween 80, 1 ml/liter; 1, 3-butylene glycol, 100 ml/liter; 85% lactic acid, 100 ml/liter; or acetone-HCl, 100 ml acetone/liter of 0.32 N HCl. In a second greenhouse study, first-year seedlings were sprayed to drip at 10 different benomyl concentrations (0.5 to 50 g active ingredients/liter), either suspended in water or mixed with Tween 20 (5 ml/liter and 10 ml/liter), Tween 80 (5 ml/liter and 10 ml/liter), or Biofilm (5 ml/liter and 10 ml/liter). Ten days after treatment, phytotoxicity was recorded and plants were harvested for bioassay. Results were expressed as the mean bioassay score of six samples from three sampling sites per tree (top, middle, bottom) for each treatment. Results of these

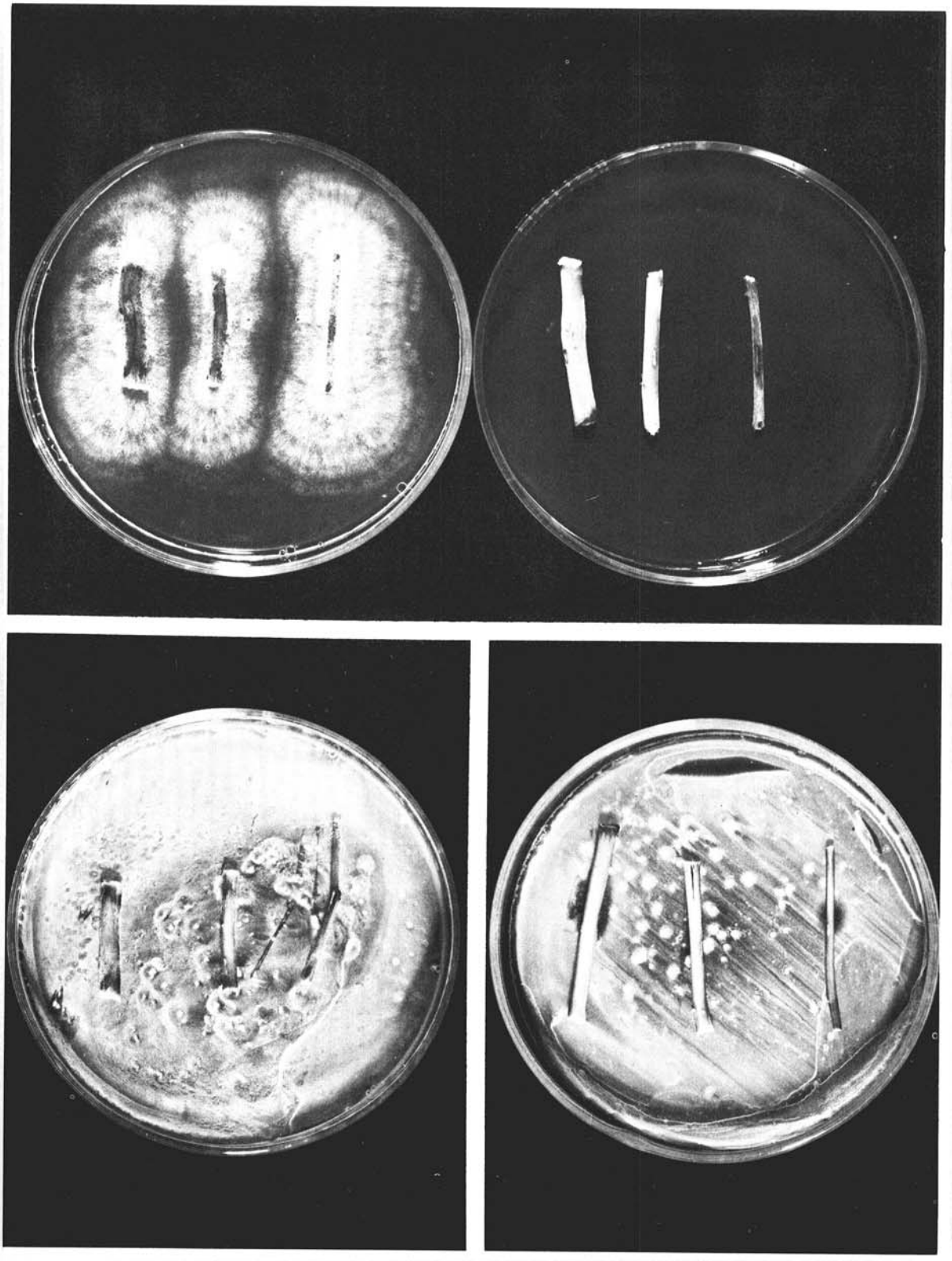

Fig. 1. Bioassay of xylem segments from 97-cm tall greenhouse-grown elms sprayed to drip with 18.1 g benomyl/liters of water. Water sprayed controls are on the left. Segments from left to right were obtained from the base, center, and top of the plants, respectively. Upper plates were inoculated by streaking a conidial suspension of *Ceratocystis ulmi* over the segment on potato-dextrose agar. Segments in the lower plates were placed directly on a freshly seeded potato-dextrose agar surface.

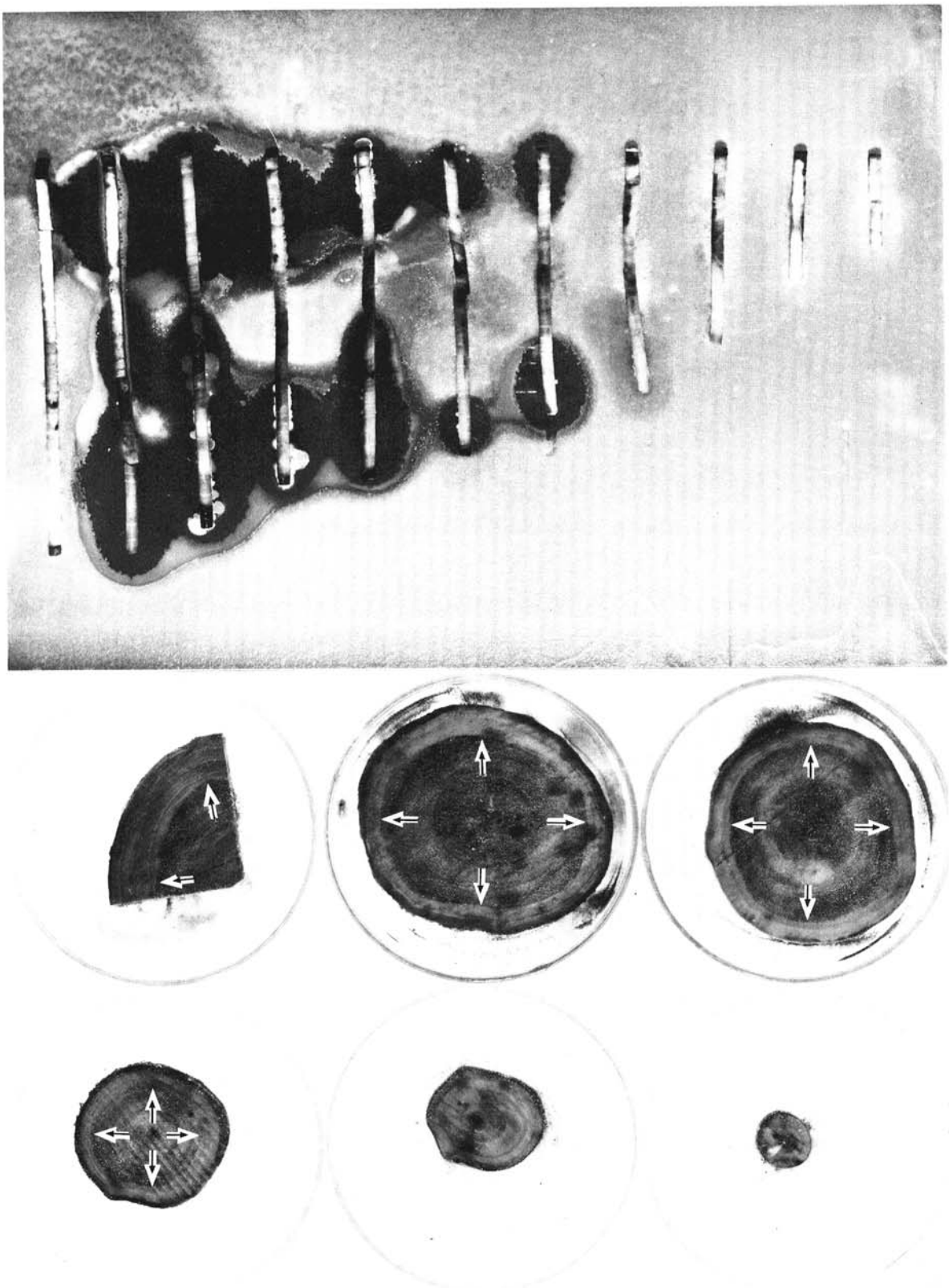

Fig. 2. Bioassay of sections taken from a 12.2 m elm treated by low pressure trunk injection with 25.0 g of benomyl solution in acetone-HCl after plating on *Ceratocystis ulmi* seeded potato-dextrose agar. Increment borings in the upper plate were taken, respectively, left to right from an untreated tree, and from 0.8, 0.9, 1.2, 1.5, 2.4, 3.0, 4.6, 6.1, 7.6, and 9.1 m above the ground in the treated tree. Cross sections in the lower plates were taken from the same treated tree 10.7, 9.1, 7.6, 6.1, 4.6, and 3.0 m above the ground. Arrows indicate margins between fungitoxic and nontoxic tissue.

tests were subjected to statistical analysis of variance and significant differences between means determined by Duncan's New Multiple Range Test (22).

In the 1971 municipal foliar spray trials, benomyl was applied at 4.8 g/liter by Milwaukee Forestry Bureau arborists with a John Bean Rotomist 100E mist blower at the rate of 8 to 12 liters/tree. One hundred and twenty blocks of trees (five trees/block) were selected in the area of treatment, and all treatments and controls at the various times of treatment were randomized throughout the treatment area. Benomyl was applied in water alone, with 0.5 ml/liter of Biofilm, and with 4.8 g/liter methoxyclor. Ten different blocks were sprayed with each formulation beginning May 19, June 3, and June 15. Ten unsprayed blocks for each time of treatment served as controls.

Trees in the 1972 Milwaukee trial were sprayed from June 6 to 28 at rates of 1.2, 2.4, or 4.8 g/liter with approximately 10 liters applied/tree. Treatments were applied to 40 randomized tree blocks (10 blocks/treatment) and each block contained approximately 50 trees. Control blocks remained unsprayed. Final readings on all protective sprays were completed in late August, the season of treatment.

Therapeutic trunk and soil injections.—Trunk injections were made using the J. J. Mauget system (J. J. Mauget Co., Burbank, Calif.) (10, 15). In 1969, benomyl was applied therapeutically from August 11 to September 12 to large lawn trees in various stages of decline from *C. ulmi* infection. In 1970, similar injections were applied between June 10 to July 28 to elms in early stages of natural infection (mostly 5% crown damage or less). Therapeutic trunk injection trials were repeated again in 1971 in the villages of River Hills and Waukesha. In Waukesha, infected branches of treated trees were removed and trees were retreated 10-12 days and 20-22 days after the original treatment. Therapeutic soil injections of benomyl in 1970 were applied between June 25 and July 28 in the root zones of elms in early stages of infection, using a standard arborist pressure root "feeder" at 3.5 kg/tree in 378 liters of water. Other infected trees were left untreated as controls.

To test the therapeutic value of the Mauget-injector-benomyl treatments under conditions of controlled inoculation with *C. ulmi*, 46 elms in River Hills were inoculated on June 4, 1971, at two points in the upper crown. In one series, trees were treated once on June 17; in a second series, trees were treated twice (May 27 and June 17); in the third series, trees were treated three times (June 16, 28, and July 9) in the year of inoculation and once on June 1, 1972 if they were still living; in the fourth series, trees were treated four times in the year of inoculations (May 27, June 16, 28, and July 12) and again on June 1, 1972. Final readings on all therapeutic treatments were completed in late August, the year after initial treatment.

Protective trunk injections.—Healthy elms treated preventively by Mauget-benomyl trunk injections in 1971 were randomly selected from an area with a previous history of high disease incidence. The first group of 64 trees were treated with a single application between May 24-26; the second 50 trees were treated on June 7-9; and the third 35 trees were treated on June 14-17. The fourth group of 36 trees

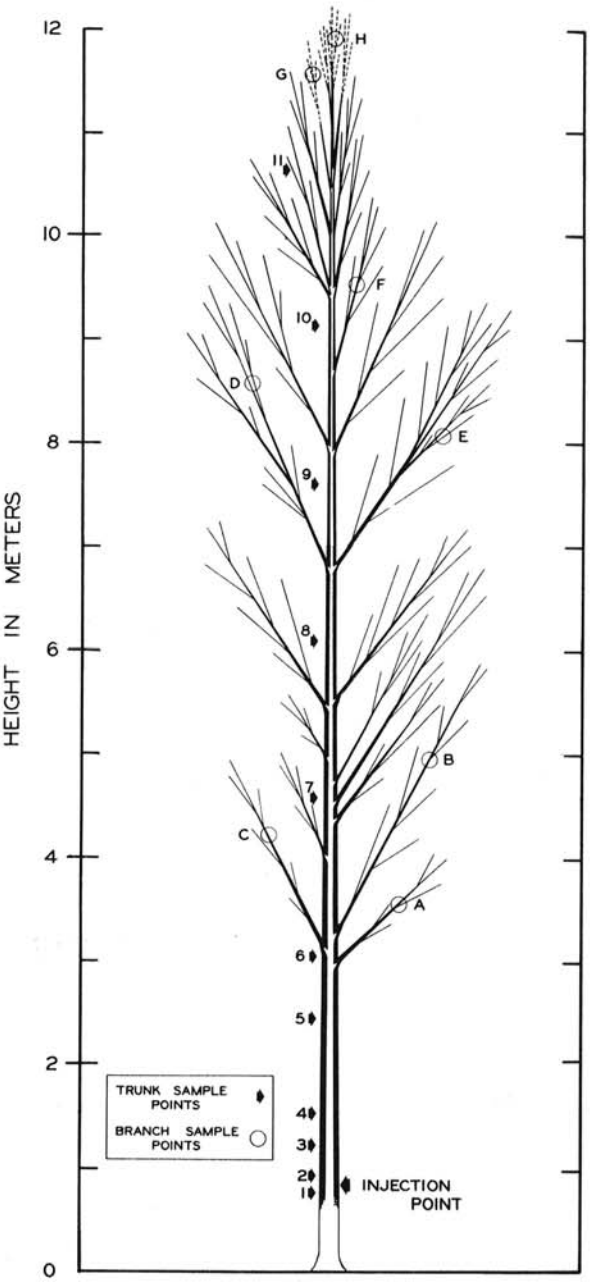

Fig. 3. Scale reconstruction of fungitoxic areas in the xylem of a 12.2 m elm treated by low pressure trunk injection with 25.0 g of benomyl solubilized in acetone-HCl. Dark areas indicate areas of activity, whereas dotted lines indicate no detectable activity. Numbers 1-11 indicate trunk sampling points bioassayed as indicated in Fig. 3. Letters A-H indicate small branch bioassay sampling points bioassayed as indicated in Fig. 2.

received a treatment on May 24-26 and again on June 14-17. The 61 control trees remained untreated. In 1972, spring trunk injections (May 25 to June 15) were applied to 688 healthy elms in the villages of Bayside, Whitefish Bay, Shorewood, and River Hills, Wisconsin. Adjacent elms remained untreated as controls. Final observations were made in late August, the year of treatment.

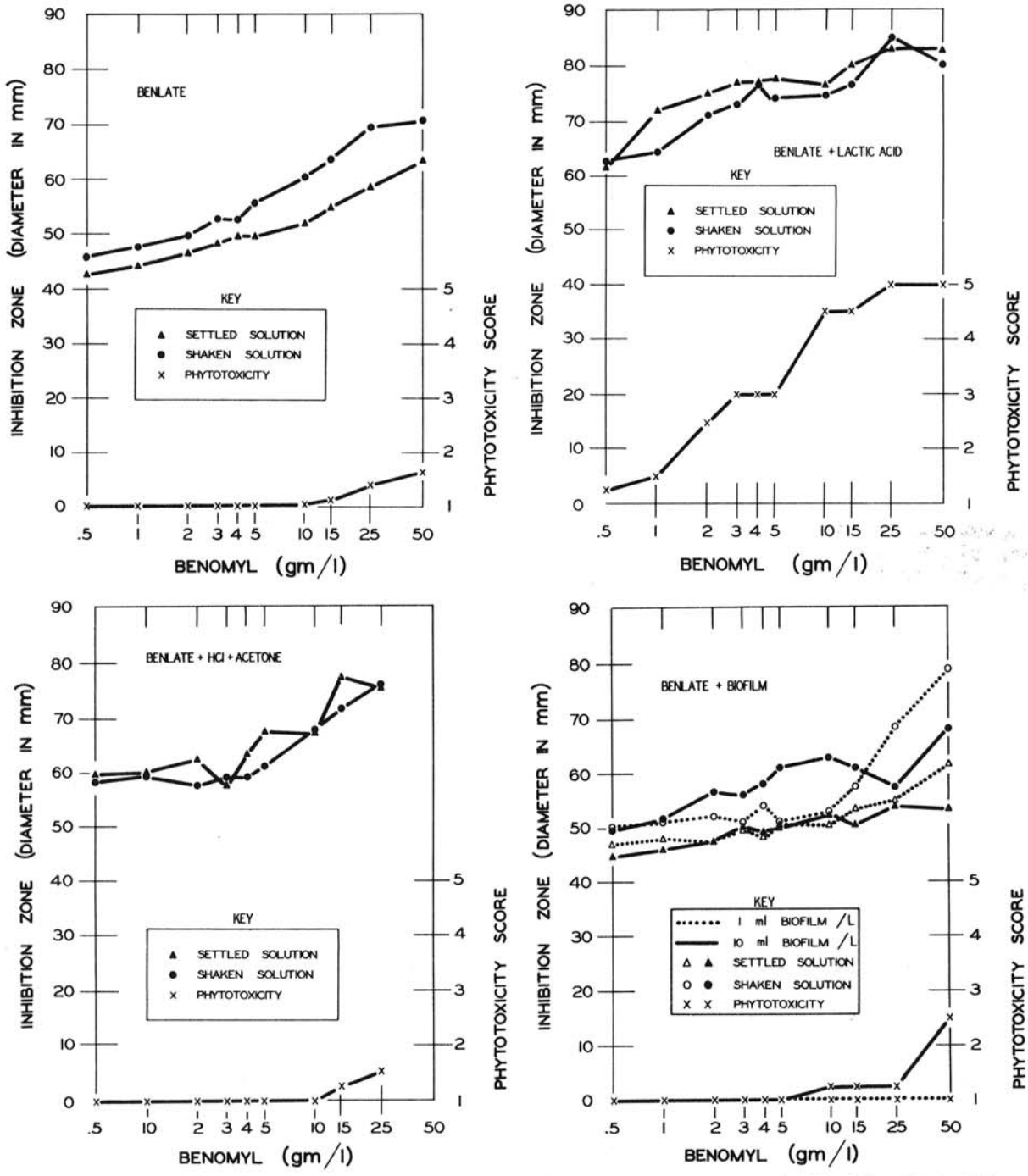

Fig. 4. Fungitoxicity and phytotoxicity of various benomyl solutions and suspensions. Fungicidal activity of test solutions was determined by the filter paper disk method (12.7-mm diam disks) after incubation at 24 C for 96 hr on potato-dextrose agar seeded with conidia of *Ceratocystis ulmi*. Phytotoxicity score on treated elm leaf strips: 1 = nonphytotoxic, 2 = slight marginal water soaking or browning, 3 = moderate marginal browning with necrotic spot, 4 = severe browning, 5 = complete necrosis.

Statistical analysis of data from preventative and therapeutic tests were by X^2 method (22). Results are expressed as the probability that the incidence of new infection, or level of remission, in established infections in treated trees is the same as that in untreated trees.

New injection procedures.—Although our municipal trials used the commercially available

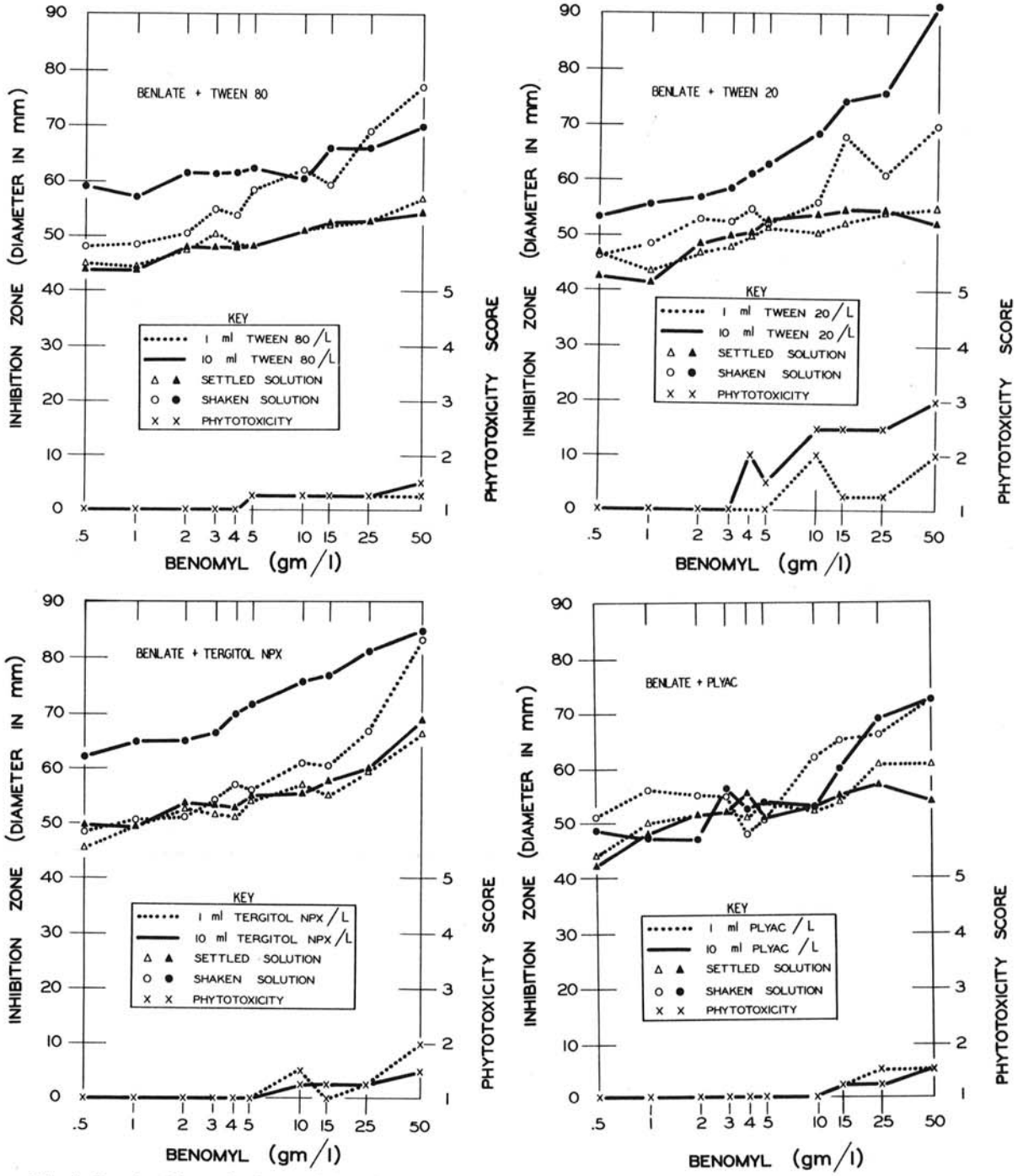

Fig. 5. Fungitoxicity and phytotoxicity of various benomyl solutions and suspensions. Fungicidal activity of test solutions was determined by the filter paper disk method (12.7-mm diam disks) after incubation at 24 C for 96 hr on potato-dextrose agar seeded with conidia of *Ceratocystis ulmi*. Phytotoxicity score on treated elm leaf strips: 1 = nonphytotoxic, 2 = slight marginal water soaking or browning, 3 = moderate marginal browning with necrotic spot, 4 = severe browning, 5 = complete necrosis.

Mauget injection system, other devices have recently become available. The SIReservoir system (Systemic Implant Reservoir Corp., Madison, Wisconsin 53705) (U.S. Patent Pending), involved drilling 1.27 cm holes, 12.7 cm apart around the trunk into which were implanted plastic capsules (4-ml capacity). These were then filled with benomyl suspensions by hypodermic needle. Creative Sales, Inc., Fremont, Nebraska, markets plastic injection devices called "Medicaps" (U.S. Patent Pending) which can be produced to contain dry benomyl mixed with "conditioners" in a hygroscopic binder (2). These are also permanently implanted in the tree trunk. These methods were evaluated for their usefulness as trunk injection systems when used with various benomyl formulations; resultant antifungal activity was measured by branch bioassays.

Of the several pressure injection systems (11, 13), only the (Elm Research Institute, Waldwick, N.J.)Low Pressure Tree Injector (Model 102) (U.S. Patent Pending) was tested in these investigations. This system used 1.27 cm holes, 5.1-cm deep, and spaced 12.7-cm apart around the tree's circumference. Special pipe T's and a single T-vent end fitting were then screwed into each hole, fitted with pressure tubing and connected to a 7.6 liter pressure tank fitted with a hand pump. This system operated without leaking at pressures of 2.1 to 2.8 kg/sq cm. Using this system, benomyl was applied to nursery elms and mature municipal trees at various concentrations and volumes (1, 5, and 25 g/liter) in water and 5 g/liter in the acetone-HCl formulation.

RESULTS.—*Laboratory evaluation of "improved" benomyl formulations.*—Benomyl was extremely soluble in lactic acid as previously reported (8, 14), and agar-diffusible antifungal activity was the greatest of all the formulations tested over a wide range of concentrations (Fig. 4). No effect of agitation was observed, indicating that all of the active component was in solution. The lactic acid formulations were, however, extremely phytotoxic to elm leaves and toxicity extended below the 0.5 g/liter dilution (Fig. 4).

Benomyl in acetone-HCl was also highly active (Fig. 4). Inhibition zones at all concentrations were larger than the benomyl-water controls, but slightly less than the benomyl-lactic acid solutions. Agitation did not affect activity. Phytotoxicity did not differ from that of the benomyl-water controls (Fig. 4).

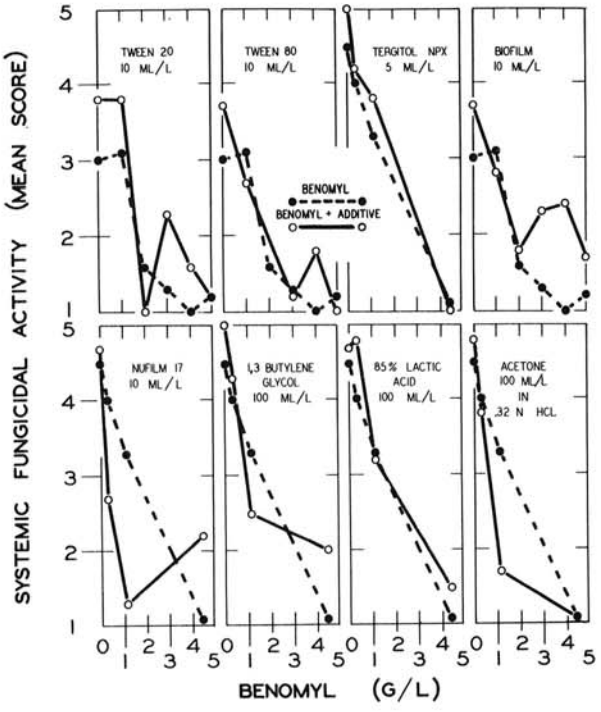

Fig. 6. Bioassay of the antifungal activity of the xylem from greenhouse elms sprayed with various benomyl formulations. Each point indicates mean bioassay scores of six bark-peeled, xylem samples (top, middle, bottom) from two plants after 10 days incubation on *Ceratocystis ulmi* seeded potato-dextrose agar plates. Systemic antifungal score: 1 = no growth of *C. ulmi* on the sterile twig; 2 = slight, slow-developing fungal growth; 3 = fair growth with limited coremial development; 4 = good growth with fair coremial development; 5 = heavy growth with intense coremial production. Antifungal scores of 1 were recorded for all benomyl spray concentrations above 5 g benomyl/liter of solution or suspension.

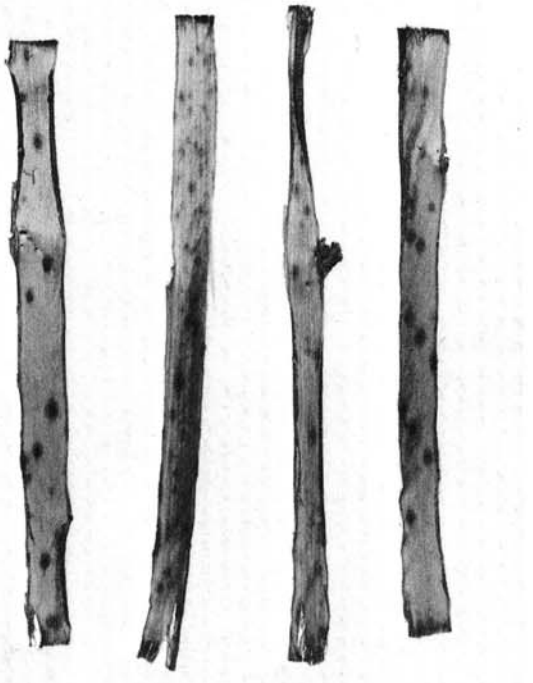

Fig. 7. Phytotoxicity of benomyl solutions in the cambial region of bark of 97-cm elms sprayed to drip with 18.1 g benomyl solubilized in acetone-0.32 N HCl. Toxicity associated with the lenticels indicates the probable route of benomyl uptake after spraying.

TABLE 1. Natural Dutch elm disease development in municipal elms treated with benomyl applied as a foliar spray[a]

Treatment dates	Benomyl conc. (g/liter)	Total blocks	Total trees	Average volume (liter/tree)	Disease incidence: Root graft or recurrent infection (No.)	(%)	New infection (No.)	(%)	
				1971 Trials					
May 19-21	4.8	10	48	7.6	1	2.1	1	2.1	
June 3-4	4.8	8	40	5.7	1	2.5	0	0.0	
June 15	4.8	7	31	9.5	0	0.0	0	0.0	
Total		25	119		2	1.7	1	0.8	$P < .005$[e]
May 21-27	4.8	9	43	7.6	3	7.0	0	0.0	
June 4	4.8[b]	9	41	5.7	0	0.0	0	0.0	
June 15	4.8	7	34	9.5	3	8.8	0	0.0	
Total		25	118		6	5.1	0	0.0	$P < .005$
May 27	4.8[c]	9	45	5.7	1	2.2	0	0.0	
June 4-10	4.8[d]	9	44	5.7	0	0.0	0	0.0	
Total		18	89		1	1.1	0	0.0	$P < .005$
All treatments	4.8	68	326	7.6	9	2.8	1	0.3	$P < .005$
Untreated controls	0.0	30	146	0.0	5	3.4	5	3.4	
				1972 Trials					
June 26-28	4.8	9	437	10.2	15	3.4	3	0.7	$P < .005$
June 13-19	2.4	7	349	8.3	9	2.6	5	1.4	$P < .250$
June 7-13	1.2[b]	10	512	7.2	29	5.7	20	3.9	$P < .500$
Controls	0.0	14	692	0.0	22	3.2	19	2.7	

[a] Benomyl sprays applied by John Bean Rotomist 100 E mist blower. Various treatment blocks randomized through the area to be sprayed.

[b] Biofilm added to spray mixture at .5 ml/liter.

[c] Methoxychlor added to spray mixture at 120 ml of 25% emulsifiable concentrate/liter.

[d] Methoxychlor added to spray mixture at 4.8 g of wettable powder/liter.

[e] Data analysis by X^2 method. P indicates probability that incidence of new infection in treated trees is the same as the incidence in untreated trees.

Addition of surfactants, with a few exceptions, did not greatly increase the size of the inhibition zones produced by the 72-hr standing suspensions, but increases in activity were observed in the shaken suspensions (Fig. 5). Agitated benomyl-Tergitol NPX (10 ml/liter) showed increased activity and was the most effective surfactant used; phytotoxicity did not differ from the controls. Tween 80 (10 ml/liter) also increased activity, particularly at the lower benomyl concentrations, without increasing phytotoxicity, and shaking greatly increased antifungal activity (Fig. 5). Tween 20 (10 ml/liter) also increased benomyl activity, but resulted in increased phytotoxicity. None of the other materials tested produced increased antifungal action (Fig. 4, 5).

Foliar applications.—In the initial trial, bioassays of xylem segments from benomyl-sprayed greenhouse elms, 10 days after treatment, indicated high level, uniformly distributed antifungal activity at concentrations from 18.1 g/liter down to 1.1 g/liter (Fig. 1, 6). Formulations of benomyl, with lactic acid, 1,3-butylene glycol and acetone-HCl (where 0.32 N HCl diluent was used instead of distilled water) were phytotoxic at all concentrations. In the second greenhouse spray trial, similar high level systemic antifungal activity was detected at concentrations down to 2 g/liter (Fig. 6). In general, additives did not greatly improve systemic antifungal activity. Tween 80-benomyl mixtures were probably slightly more active than the rest; they produced inhibition zones around the xylem segments at concentrations above 3 g/liter. None of the surfactant-benomyl sprays were phytotoxic.

Benomyl formulations (particularly with lactic acid or acetone-0.32 N HCl produced a characteristic pattern of bark toxicity. On the inner side of the peeled bark, brown necrotic spots were scattered uniformly over the cambial surface adjacent to the lenticel openings (Fig. 7). This suggested the possibility that the major route of uptake of the benomyl sprays was through the bark lenticels, rather than through the leaves.

Prevention of Dutch elm disease was achieved in 1971 in large municipal elms by foliar mist-blower applications of benomyl suspensions at 4.8 g/liter (Table 1). No trees sprayed with benomyl-Biofilm or

TABLE 2. Remission of Dutch elm disease in naturally infected municipal elms treated with benomyl[a]

Method of treatment	Locality	Crown damage (%)	Year of treatment	Dates of treatment: Before June 25 Total trees	Before June 25 Trees remaining healthy (No.)	Before June 25 Trees remaining healthy (%)		Dates of treatment: After June 25 Total Trees	After June 25 Trees remaining healthy (No.)	After June 25 Trees remaining healthy (%)	
Trunk injection											
	River Hills[b]	> 5	1969					27	1	3.7	$P < .750$[g]
	River Hills[c]	> 5	1970	5	0	0		1	0	0.0	
	River Hills[b]	< 5	1969					19	10	52.6	$P < .025$
	River Hills[c]	< 5	1970	19	2	10.5	$P < .750$[g]	18	11	61.1	$P < .005$
	River Hills[f]	< 5	1971	40	3	7.5	$P < .100$	49	25	51.0	$P < .010$
	Waukesha[f]	< 5	1971	13	9	69.2	$P < .025$	2	2	100.0	$P < .005$
Soil injection											
	River Hills[d]	< 5	1970					9	6	66.7	$P < .025$
Untreated controls											
	River Hills and Waukesha[e]	< 5	1970 & 71	27	2	7.4		12	1	8.3	

[a] Elms with complete remission of disease remained symptomless at least 15 months following treatment.

[b] Trees injected with benomyl suspensions (1 to 10 g/liter of distilled water) applied in Mauget injection tubes (0.8-ml capacity) at (5- to 8-cm) intervals around the circumference of the trunk. Tubes were filled several times with benomyl solutions. Treatments applied in Village of River Hills from 8/11 to 9/12/69.

[c] Benomyl suspensions (2.5 gm active ingredient/liter) were applied by trunk injections with 10-ml Mauget feeder tubes and cups, and repeated at 10-day intervals depending upon the progress of the disease.

[d] Benomyl was applied to the root zone by means of a standard pressure tree feeder, at 18.1 g/liter of water.

[e] Control trees were similar to treated trees, but were left untreated to determine natural progress of disease.

[f] Benomyl suspensions (2.5 gm active ingredient/liter) were applied by trunk injections with 65-ml Mauget feeder tubes and cups, and repeated twice at 10 day intervals depending upon the rates of disease development. Infected branches pruned to first branch below detectable xylem discoloration.

[g] Analysis of data by X^2 method. Results expressed as probability that the level of remission in treated trees is the same as that in the untreated trees.

benomyl-methoxychlor developed new infections, while untreated controls had 3.4% infection. Though incidence of disease was low, control was statistically significant. The more extensive municipal foliar spray trial of 1972 produced essentially the same results (Table 1). Benomyl (plus 0.5 ml/liter of Biofilm) applied at 0, 1.2, 2.4, and 4.8 g/liter of mist-blown spray resulted, respectively, in 2.7, 3.9, 1.4, and 0.7% incidence of new infections.

Prevention and therapy by soil or trunk injection.—Therapeutic treatment of early-stage (*C. ulmi*) infections by soil application of benomyl were surprisingly effective (Table 2). Surviving trees treated in this manner looked darker green than normal, and a full season after treatment appeared generally healthier than surrounding noninfected elms.

Prevention and therapy of Dutch elm disease was also achieved by benomyl trunk injections using Mauget injectors. The first therapy trials, without controls, using naturally infected elms, suggested effectiveness only where infections were quite limited at the time of treatment (Table 2). If the disease had progressed to more than 5% crown damage, almost all the trees were dead by the end of the season following treatment. Later, more extensive therapy injection trials confirmed preliminary findings, but indicated that the most effective time period for treatment in Wisconsin was probably after June 25, when the new bark beetle-induced branch infections were appearing. Pruning of infected branches combined with benomyl injections, however, were effective in curing many of the infected elms, even those detected before June 25. Early, rapidly developing, infections such as occur with root graft transmissions or recurrent disease from previous year infections were clearly not controlled by the trunk injections (Table 2). In therapy trials using artificially inoculated municipal elms treated by trunk injection three times during the season of inoculation and again the following spring, 83% remained healthy, while only 20% of the controls survived (Table 3).

Protective treatments in 1971 of healthy municipal elms by Mauget trunk injections of benomyl reduced the incidence of new infections from 16.4% in the controls to 6.5% in the treated trees (Table 4). Times of application did not affect the results consistently, although the latest treatment (June 14) had the fewest new infections. In the similar, but larger trial in 1972, untreated elms developed over 3 times as many new infections as did treated trees (Table 4).

New injection procedures.—Of various water suspensions of benomyl applied via the SIReservoir system, only those applied at the highest dose (45 g/liter and 162 ml/tree) could be detected in bioassays of branches from treated trees 1 week after treatment (Table 5). Activity, however, was low and none could be detected at the later sampling times. Of the other formulations, the highest activity

TABLE 3. Dutch elm disease development in artificially inoculated municipal elms treated therapeutically in 1971 with benomyl applied by trunk injection[a]

Treatment times	Number of trees	Total active ingredient applied (Total gm/tree)	Trees remaining healthy (No.)	Trees remaining healthy (%)	
June 17	5	2.09	3	60.0	$P < .250$[c]
May 27, June 17[b]	9	3.54	4	44.4	$P < .500$
June 16, 28, July 9	12	6.39	10	83.3	$P < .005$
May 27, June 16, 28, July 12[b]	10	6.85	6	60.0	$P < .100$
Total treated	36	5.21	23	63.9	$P < .250$
Untreated controls	10	0.00	2	20.0	

[a] Each treated tree was injected with a suspension of benomyl (2.5 g/liter) at 5- to 8-cm intervals around the trunk using 65 ml Mauget cups. Trees ranged from 22 to 30 cm d.b.h. Control trees remained untreated. Trees were inoculated at two points in the upper crown on June 4, 1971 (approx. 10^6 conidia/ml).

[b] All symptomless trees retreated June 1, 1972.

[c] Analysis of data by X^2 method. Results are expressed as probability that the level of remission in treated trees is the same as that in the untreated trees.

1 week after treatment was observed in samples from the high dose acetone-HCl and Biofilm formulations. Low order activity in the Biofilm treatments was detectable for more than 3 weeks. No activity was detected in the Medicap treated trees, and one of the two treated trees developed a new *C. ulmi* infection.

With the Mauget injector system using the 65 ml cup, moderate activity could be detected for more than 1 week using the water suspension. Low order activity could be detected over 4 weeks with the acetone-HCl and the Biofilm formulations (Table 5).

Using the E.R.I. low pressure application system, moderate antifungal activity was detected, in small branches, for over 2 weeks when applied in a water suspension of 1 g/liter and 7 liters/tree, but at lower doses, no activity could be detected. Activity, however, was detectable in the lower trunks of all trees treated with benomyl-water suspensions at all doses, but distribution in the upper branches was only erratically detectable. Clearly, the greatest activity was obtained with acetone-HCl solubilized-benomyl applied by the E.R.I. pressure

TABLE 4. Incidence of Dutch elm disease in municipal elms treated protectively by trunk injection with benomyl[a]

Locality of treatment	Number of applications	Date	Number of trees	Disease incidence: Root graft or recurrent infection (No.)	Root graft or recurrent infection (%)	New infection (No.)	New infection (%)	
			1971 trials					
River Hills	1	May 24-26	64	2	3.10	4	6.30	$P < .050$[b]
River Hills	1	June 7-9	50	1	2.00	3	6.00	$P < .100$
River Hills	1	June 14-17	35	2	5.70	2	5.70	$P < .250$
River Hills	2	May 24; June 14	36	2	5.60	3	8.30	$P < .500$
Total treated			185	7	3.80	12	6.50	$P < .005$
Total untreated			61	2	3.30	10	16.40	
			1972 trials					
Bayside	1	May 25-June 15	270	8	2.97	0	0.00	
Whitefish Bay	1	May 25-June 15	112	1	0.89	1	0.89	
Shorewood	1	May 25-June 15	174	7	4.02	1	0.57	
River Hills	1	May 25-June 15	132	5	3.79	0	0.00	
Total treated			688	21	3.05	2	0.15	$P < .250$
Bayside	0	Untreated	143	5	3.50	0	0.00	
Whitefish Bay	0	Untreated	178	7	3.93	0	0.00	
Shorewood	0	Untreated	157	6	3.82	3	1.91	
River Hills	0	Untreated	141	6	2.71	3	2.13	
Total untreated			619	24	3.88	6	0.97	

[a] Each treated tree was injected with a suspension of benomyl (2.5 gm/liter) at 5- to 8-cm intervals around the trunk using 65-ml Mauget cups.

[b] Data analysis by X^2 method. P indicates probability that incidence of new infection in treated trees is the same as the incidence in untreated trees.

TABLE 5. Bioassay of systemic fungicidal activity in the xylem of elm branches from large elms following trunk injections with benomyl[a]

Type of injector	Solvent or additive concn.	Benomyl concentration (g/l)	Volume applied (ml/tree)	Number of trees	Systemic fungicidal activity[b]	
					Period of detectable activity (weeks)	Highest activity detected
SIReservoir[c]	H_2O	1.0	144	2	< 1	0
SIReservoir[c]	H_2O	5.0	150	2	< 1	0
SIReservoir[c]	H_2O	45.0	162	2	> 1 < 3	+
Mauget	H_2O	2.5	780	2	> 1 < 4	+++
SIReservoir[c]	Acetone-HCl	5.0	156	2	> 1 < 2	++
SIReservoir[c]	Tween-20 (5 ml/liter)	5.0	150	2	> 1 < 2	++
SIReservoir[c]	Tween-80 (5 ml/liter)	5.0	144	2	< 1	0
SIReservoir[c]	Biofilm (5 ml/liter)	5.0	138	2	> 3	++
Mauget	Acetone-HCl	5.0	725	1	> 4	+
Mauget	Tween-20 (5 ml/liter)	5.0	80	1	> 4	+
Mauget	Tween-80 (5 ml/liter)	5.0	170	1	< 4	0
Mauget	Biofilm (5 ml/liter)	5.0	240	1	> 4	+
ERI-Pressure	H_2O	1.0	7,000	1	> 2	+++
ERI-Pressure	Acetone-HCl	5.0	5,000	1	> 1	++++
Medicap	Not specified	Unknown		2	< 1	0
All controls	No treatment	0.0	0.0	9	< 1	0

[a] Treated elms were mostly mature trees (approx. 50 cm d.b.h.) in municipal situations. Treatments were made in June, 1972 and upper branch samples collected at weekly intervals up to 4 weeks.

[b] Systemic fungicidal activity of xylem branch segments on *Ceratocystis ulmi* seeded potato-dextrose agar plates after 2 weeks incubation: 0 = none, + = slight (25% greater than controls), ++ = moderate (50% greater than controls), +++ = high (75% greater than controls), ++++ = completely fungitoxic. Activity values are means from four to seven twig samples/branch selected randomly from two to five different places in the upper tree crown.

[c] All SIReservoir treated trees were injected three times in the same reservoir during a 24-hr period to achieve the indicated volume.

system. In one case, 25 g (5 g/liter and 5 liters/tree) was injected into a 12.2-m elm tree in less than 30 minutes. Bioassays of this tree when cut and dissected 3 days after treatment indicated high antifungal activity throughout the tree's functioning xylem (Fig. 2, 3).

DISCUSSION.—*Activity of formulations.*—McWain & Gregory (14), stated that benomyl solubilized in lactic acid seemed to be the most promising formulation for use in elm tree injections. Our studies confirmed their findings regarding the solubilization of benomyl in lactic acid. However, the extreme phytotoxicity of these preparations at almost all dilutions in our tests would seem to preclude the practical use of lactic acid as a solubilizing agent.

Acetone-HCl-solubilized benomyl, while having in vitro activity almost as great as the lactic acid-solubilized benomyl was no more phytotoxic than the benomyl-water controls. Stock solutions when diluted to 5 or 10 g active ingredient/liter with distilled water produced highly active nonphytotoxic solutions which appeared to be ideal for trunk injection.

None of the surfactants in our in vitro tests, was as effective in increasing benomyl activity as the acetone-HCl preparations.

Bioassays and foliar sprays.—The antifungal effects on *C. ulmi* of xylem segments from greenhouse-grown elms sprayed with various benomyl formulations were quite remarkable. The high level of the systemic antifungal effects remaining would suggest a long residual action of the active compound in the xylem.

The bioassay suggested that benomyl or its metabolites exist and move systemically in two different states in the xylem of treated trees. Benomyl moves passively in the free water so that in tissue samples where concentrations of the active product are high enough, inhibition zones are produced in *C. ulmi*-seeded cultures (1, 17, 18, 19, 20). The active product may also be bound in the living xylem tissues and not released to produce inhibition-zones, although growth of *C. ulmi* on this tissue is prevented. Erwin et al. (7) suggested that ^{14}C-thiabendazole binds with higher molecular weight plant products, which prevents diffusion of the material into the agar. These effects probably explain the failures of various authors (9, 21) to detect inhibition zones using branches from elms protected from disease by benomyl.

The uniformity of fungitoxic activity throughout the xylem of the greenhouse-sprayed plants was surprising if one assumed that uptake of the product was mainly through the leaves, since the lower parts (lower 10-15 cm) of most of the treated elms were leafless. The observation in several of the phytotoxic formulations of brown, necrotic spots in the cambial

region under each lenticel suggests that uptake of the product from foliar sprays is primarily through the lenticels, and bark expansion cracks permitting close contact of the product with living tissue. Prasad's (20) difficulty in achieving benomyl uptake through elm leaves would also suggest something other than a foliar route of uptake.

The use of foliar sprays as a means of mass application of benomyl in control of Dutch elm disease may have practical potential. Large scale application of benomyl to municipal elms with mist blowers during two seasons have confirmed protective results of the greenhouse spray trials. Hart (9) obtained similar protection by repeated applications over a 2-year period. The municipal trials indicated the need for relatively high concentrations (4.8 g/liter) of benomyl to achieve practical protection. At the current price of Benlate, the cost of spraying a large elm tree, exclusive of labor costs, would be slightly over $3.00. Bioassays of branches of selected muncipally sprayed trees indicated erratic distribution of the benomyl. Although practical protection was achieved by these foliar sprays, the actual coverage achieved was poor. Mist-blown sprays, as presently produced commercially, apparently failed to achieve the uniform wetting produced at similar doses on greenhouse-sprayed plants. If lenticel uptake is the major route of benomyl movement into the tree, the importance of good coverage with complete surface wetting cannot be overstated. Large hydraulic sprayers would probably be the better choice of equipment where elm trees are not too large.

Trunk injections.—Prevention of Dutch elm disease in municipal elms by protective trunk injection significantly reduced incidence of new infections, but had no effect on root graft transmission. The relatively high labor costs in the simplest of such treatments may exclude these methods for use as mass municipal treatments. In the hands of the skilled arborists, however, custom treatments for individual tree owners could be of immense value in reducing losses from this disease.

For protective treatment, the Mauget system appears presently to be the technique of choice, because of the large volumes which can be applied, as well as the small injection holes produced. This system, however, has several disadvantages, such as the long time period required to complete the injection and the dependence upon weather conditions for optimum uptake. Use of acetone-HCl solutions to replace benomyl suspensions would apparently improve the effectiveness of this technique, but municipal trials will be needed to confirm conclusions based on bioassay data.

Therapeutic treatments by Mauget injection were moderately to highly successful. Such treatments appear to be the first large scale cures effected on otherwise doomed American elms. Retreatment during the season of detection and the following year increased the numbers of surviving individuals; pruning of infected branches further increased the number of survivors. The necessity of retreatment for effective cures might be lessened through the use of devices such as the E.R.I. low pressure injector. Although its value in curing infected trees remains to be proven, bioassays of treated trees indicate high potential particularly with the acetone-HCl formulation. The low cost and simplicity of design of this product, and reduced health hazards compared to use of high pressure injection are advantages of this injector. Initial remission (through one season) of treated infected trees having over 10% crown damage with the E.R.I. device suggest its usefulness for therapy of more advanced disease cases.

Results of the laboratory, greenhouse, and municipal experiments using various techniques of application indicate that considerable benefit was derived from the use of benomyl. Foliar sprays were particularly promising from an economic point of view. Therapeutic soil and trunk injections saved many otherwise doomed elms. When combined with adequate sanitation programs, and measures to prevent root graft transmission, the recommendation of certain of these benomyl application methods for practical control of Dutch elm disease seems indicated.

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