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Disease Control and Pest Management

Distribution and Retention of Thiabendazole Hypophosphite and Carbendazim Phosphate Injected into Mature American Elms

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ABSTRACT

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When exposed root flares were injected with solutions of thiabendazole hypophosphite and carbendazim phosphate, the fungicides became completely and evenly distributed in all of the outer sapwood in the crown of mature American elms (*Ulmus americana*) provided the dose was adequate. Carbendazim phosphate applied at 0.98 and 1.95 g a.i. per centimeter of trunk diameter and thiabendazole hypophosphite applied at 1.86, 3.72, 5.59, and 8.94 g a.i. per centimeter of diameter apparently protected mature elms from Dutch elm disease during the year of injection. Only thiabendazole hypophosphite applied at 5.59 and 8.94 g/cm of diameter was detected by bioassay in the newest radial wood during the second and third growing seasons after injection. At a rate of 5.59 g/cm, thiabendazole hypophosphite applied at a concentration of 3.0 g/L and volume rate of 1.86 L/cm of diameter apparently maximized distribution and retention of the fungicide.

Additional key word: Ceratocystis ulmi.

Dutch elm disease (DED), a vascular wilt disease of elms caused by the fungus *Ceratocystis ulmi* (Buism.) C. Moreau, has destroyed a large proportion of one of North America's most important shade trees. The earliest attempts to control DED in American elms with systemic chemicals took place at the Connecticut Agricultural Experiment Station (5,6,22). Oxyquinoline benzoate showed the greatest promise, but only by reducing the rate at which trees died. Dimond and Stoddard (6) recognized that injection site injury could be significant, that obtaining adequate chemical distribution throughout the tree was difficult, and that better chemicals were needed. To be effective, any systemic fungitoxicant must be delivered uniformly to susceptible tissues in adequate concentrations and remain there for a sufficient period of time without seriously injuring the tree. In addition, Dimond et al (5) found that the chemotherapeutic dose should be proportional to the diameter of the tree and that peripheral distribution of trunk-injected chemicals could be improved by increasing the number of injection holes.

The discovery of the systemic fungicidal activity of several substituted 2-aminobenzimidazoles (9) led to the development of a number of useful compounds. Among them are thiabendazole (2-(4-thiazolyl)-benzimidazole), a broad-spectrum anthelmintic (2) and systemic fungicide (8); benomyl (methyl-1-butylcarbamoyl)-2-benzimidazolecarbamate) (4); and the hydrolytic breakdown product of benomyl, methyl-2-benzimidazolecarbamate (carbendazim), which is primarily responsible for the fungitoxicity of benomyl (3). There are several other systemic fungicides that depend on the benzimidazole ring for their fungitoxicity, and some have promise for the control of DED (13,14,19).

The objective of this research was to evaluate several different combinations of injection techniques, commercially available chemicals, and dosage rates for uniformity of distribution and retention of the fungitoxicant in as much of the aboveground outer sapwood of large American elms as possible. A chemical and dosage rate that would provide protection from *C. ulmi* for two or more growing seasons would minimize both the cost of repeated injections and the damage to the tree's vascular system. As important, however, is the fact that long-term fungitoxic activity in the newest wood is necessary for a successful therapeutic treatment because the probability of inhibiting the pathogen will be much greater if the fungitoxicant can move into new wood as the tree grows.

MATERIALS AND METHODS

Fungicides. The chemicals evaluated were thiabendazole hypophosphite (2-(4-thiazolyl)-benzimidazole hypophosphite; TBZ-P), Arbotect 20-S; and carbendazim phosphate (methyl-2-benzimidazolecarbamate phosphate; MBC-P), Lignasan BLP.

Trees. Fifty-five American elms were located on the Minnesota State Fairgrounds, St. Paul; they were reasonably uniform in trunk diameter, crown size, and condition and were not visibly infected (mean diameter = 56.9 ± 6.7 cm). Ten trees on the Minneapolis campus of the University of Minnesota were used for

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evaluation of the two carbendazim phosphate label rates. On 1 June 1977, the 55 elms were placed in 11 similar groups of five, and each group was randomly assigned a treatment.

Injection. The injections were started 15 June and completed 22 June. Information gathered for each tree at the time of injection included general condition, number of injection holes, approximate ambient air temperature, relative humidity and wind speed, and total time of uptake when possible.

Two methods were used for injecting TBZ-P. One group of five trees was trunk injected at the maximum label preventive rate and another group of five was trunk injected at the maximum label therapy rate. All other treatments for both fungicides involved groups of five trees and, except for MBC-P label rates, involved injection into exposed root flares.

For the trunk injections, the prescribed amount of solution was injected into the main stem 1 m above ground under pressure of 0.7 kg/cm^2 (10 psi). The injection holes were 7.94 mm (5/16 in.) in diameter, 2.5–3.8 cm deep, and 15 cm apart for an average of 0.22 holes per centimeter of trunk diameter. The plastic injection heads were tapered.

For root flare injection, the soil was removed for 60–90 cm from the base of the tree and to a depth of 20–30 cm. The solution was injected into holes 11.11 mm (7/16 in.) in diameter drilled 2.5–3.8 cm into the sapwood 10–20 cm apart. In 1978, brass injection heads 6.75 mm (17/64 in.) in diameter were used. Injection hole spacing was widest on wide-spreading buttress roots and closer together where there were no root flares, averaging 0.89 holes per centimeter of trunk diameter (Fig. 1). The injection holes were drilled with a high-torque electric drill equipped with a Greenley 177 spur bit or a Cleveland high-helix, high-speed metal bit. The injection pressure usually varied from 0.35 to 0.7 kg/cm^2 (5–10 psi). If a tree remained connected to the delivery tank overnight, the pressure was released and the solution permitted to flow in hydrostatically. St. Paul city water was used, and no problems with precipitation of the active ingredients were encountered.

Dosage rates. Dosage was expressed as grams of active ingredient per centimeter of tree diameter at 1.4 m above the ground. The dosage was determined by the grams per liter and liters of solution injected.

Based on other studies (12,13), 13 combinations of chemical, dosage, and injection techniques were evaluated (Table 1). The phosphate moiety was included in calculating the active ingredient rates for the MBC-P treatments, but only the thiabendazole moiety was included in the rates for the TBZ-P treatments.

Bioassays. To determine the relative strength of the chemical activity and uniformity of chemical distribution in the crowns of the treated trees, branch samples from each tree were bioassayed. A 17-m bucket truck was used to take a minimum of 16 samples at random from the ends of branches in the periphery of each tree crown. Each sample consisted of a living branch about 12 cm long with an average diameter without bark of 9.0 mm. The first samples were taken 13–15 July, 20–29 days after injection. Bioassays of trees that had significant levels of activity were repeated in July of subsequent years until only low activity was detected.

Additional trees that were root-flare injected for reasons not directly related to this study also were bioassayed, some of them several times. Most of these trees were on the Minnesota State Fairgrounds, but they were treated at different times in different years and varied widely in age and size. The data obtained from these supplemental trees provided a more complete indication of how distribution varied with time.

Bioassays used an aggressive strain of *C. ulmi*. The inoculum for bioassays was produced by shaking a small plug from an agar culture of the aggressive strain of *C. ulmi* in 10 ml of liquid nutrient medium for 36–48 hr. The resulting spore suspension was diluted to 10^6 cells per milliliter with sterile distilled water. The nutrient medium (suggested by Dr. E. S. Kondo) consisted of 20 g of dextrose, 2.0 g of L-asparagine, 1.5 g of KH_2PO_4, 1.0 g of $MgSO_4 \cdot 7H_2O$, 0.15 mg of $FeCl_3 \cdot 6H_2O$, 0.1 mg of $ZnSO_4$, 0.18 mg of $MnCl_2 \cdot 4H_2O$, 1.0 mg of thiamine HCl, 1.0 mg of pyridoxol phosphate, and 1 L of distilled water.

Petri plates containing freshly prepared 2% water agar amended with cycloheximide (200 ppm) and streptomycin sulfate (100 ppm)

TABLE 1. Distribution, strength, and retention of thiabendazole hypophosphite (TBZ-P) and carbendazimphosphate (MBC-P) in American elms

Treatment	Chemical	Label equivalent	Injection technique	Conc. (g/L)	Volume (L/cm DBH)	Active ingredient (g/cm DBH)	Sampling time (mo)	Distribution (%)	Samples with * (%)[a]	Samples with IZ (%)[b]
I	TBZ-P	Prev	ERF[c]	5.0	0.186	0.931	1	17.3 a[d]	0	0
II	TBZ-P	Ther	ERF	5.0	0.372	1.86	1	55.0 b	0 a	0
							13	15.0 m	0	0
III	TBZ-P	Prev	Trunk	5.0	0.186	0.931	1	11.3 a	0	0
IV	TBZ-P	Ther	Trunk	5.0	0.372	1.86	1	68.8 cd	27.5 b	0
							13	13.8 m	0	0
V	TBZ-P	Ther	ERF	1.0	1.86	1.86	1	62.5 bc	0 a	0
VI	TBZ-P	2×[e]	ERF	2.0	1.86	3.72	1	97.5 e	52.5 c	3.8
							13	66.3 n	0	0
							25	38.3 x	0	0
VII	TBZ-P	3×	ERF	3.0	1.86	5.59	1	97.5 e	67.5 d	12.5
							13	86.3 o	11.3 m	1.3
							25	72.5 z	0	0
VIII	TBZ-P	3×	ERF	1.5	3.72	5.59	1	78.8 d	20.0 b	1.3
							13	81.3 o	10.0 m	1.3
							25	56.3 y	0	0
XIII[f]	TBZ-P	4.8×	ERF	3.0	2.98	8.94	1	100.0 e	91.3 e	65.0
							13	97.5 p	45.6 n	19.0
							25	56.3 y	5.0 x	2.5
IX	MBC-P	3×	ERF	0.438	2.23	0.979	1	76.2 d	1.2 a	0
X	MBC-P	6×	ERF	0.875	2.23	1.95	1	100.0 e	37.8 b	3.7
							13	93.8 k	0	0
XI	MBC-P	Prev	Ground level	0.219	0.745	0.163	5	0	0	0
XII	MBC-P	Ther	Ground level	0.438	0.745	0.326	5	0	0	0

[a] Complete inhibition of synnemata over entire cylindrical surface of disk.
[b] Inhibition zones on agar around disk.
[c] Exposed-root-flare injection.
[d] Means within columns followed by same letter(s) are not significantly different ($P = 0.05$) according to LSD test.
[e] Two times therapy label rate.
[f] Added to study in 1978.

were seeded with 1 ml of the spore suspension spread uniformly on the agar surface. The plates were left uncovered until no surface moisture remained. This technique is similar to that used by Smalley et al (20).

Bark was removed from branch samples to be bioassayed, and disks 7–8 mm thick were cut from the middle of each sample and placed on seeded agar plates. Four samples were placed in each plate (four plates for each tree) and incubated at room temperature for 4–6 days. After synnemata had developed on all surfaces of wood disks from untreated trees, the inhibition of synnemata on the disks from treated trees was scored (Fig. 2). A score of 0 indicated development of synnemata over the entire disk, 1 indicated inhibition of synnemata on the top of the cylindrical surface of the disk, and 1* indicated complete inhibition of synnemata over the entire cylindrical surface of the disk. Sometimes the disks rated 1* had an inhibition of growth on the agar adjacent to the disk. The average width of this inhibition zone (IZ) was recorded. The percentage of samples with inhibitory activity (percentage distribution) was calculated as 100 × number of samples scored 1 or 1* ÷ total number of samples. The percentage of samples (disks) scored * and the percentage of samples resulting in an IZ also were calculated.

Comparisons were made by employing a least significant difference method of comparing means modified for a binomial distribution. It was assumed that at any given time after treatment, the probability that the fungicide would be detected in a given branch was equal for all branches on all trees in that treatment group.

RESULTS

Treatments I–V: TBZ-P injected at label rates. In spring 1982, Merck and Co. was granted a new federal label by the U.S. Environmental Protection Agency providing for a dosage rate three times as high as the original label had allowed. All treatments mentioned in this paper are discussed on the basis of the provisions of the original label.

When TBZ-P was root-flare (I) and trunk (III) injected in accordance with the highest preventive label rates, very low levels of chemical were detected and the trees were not resampled (Table 1). The 1977 (1-mo) bioassays indicated no difference in activity associated with differences in the volume of water used to inject the chemical at the therapy label rate (II and V). Percentage distribution and percentage of samples scored * were significantly greater with trunk injection (IV) than with root-flare injection (II) at the same dose and volume (Table 1). In 1978, only those trees that were trunk (IV) and root-flare (II) injected with the maximum therapy label rate were resampled. The levels of activity were about the same a year after injection as they were after 30 days with the preventive rate (I). After 13 mo, there was no longer a difference in the level of activity in these two groups.

Fig. 1. Exposed-root-flare injection using about one injection site per 1.25 cm of diameter (two sites per inch).

Treatment VI: TBZ-P injected at two times the therapy label rate. Complete distribution of TBZ-P was achieved with root-flare injection at the 2× rate (Table 1). Distribution data at 13 and 25 mo showed that substantial fungitoxic activity extended into the newest radial wood in the second and third growing seasons. Only two of the five original trees in this group were sampled after 25 mo because the other three were artificially inoculated in 1978, 13 mo after injection, and each of them became infected in 2 of 10 inoculated branches. All three inoculated trees were treated therapeutically in 1978 and were therefore unavailable for the 25-mo sampling in 1979.

Treatments VII and VIII: TBZ-P injected at three times the maximum therapy label rate. One month after injection, both the 2× rate applied at 2.0 g/L (VI) and the 3× rate applied at 3.0 g/L (VII) provided more complete distribution and stronger activity than the 3× rate applied at 1.5 g/L (VIII). When the trees that received the 3× rates were resampled 13 mo after injection, there were no longer significant differences in percentage distribution or percentage of samples with * between them, but the 3× rate applied at 1.5 g/L provided significantly better distribution than the 2× rate. When the trees were sampled after 25 mo, the rate of 3.0 g/L provided significantly better distribution than that of 1.5 g/L.

The bioassay data obtained from healthy and diseased elms that were root-flare injected with the 3× rate (3.0 g/L) during the course of this research are summarized in Table 2 and Figure 3. For the most part, the data in Table 2 are consistent with data for the 3× rate collected from this study alone (Table 1).

Treatment XIII: TBZ-P injected at 4.8 times the maximum therapy label rate. This treatment was included in the study in 1978 to determine whether higher quantities of the fungitoxicant would cause injury or would significantly extend the period of effectiveness. The five trees treated at the 4.8× rate sustained no visible injury. However, when trees with many epicormic branches close to the ground were treated this way, a few leaves on these

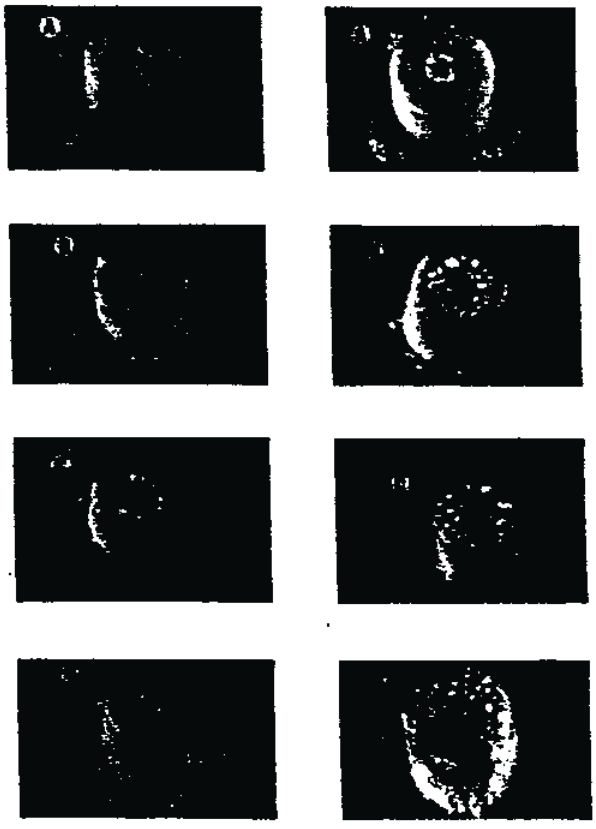

Fig. 2. Bioassay evaluation scheme: A = inhibition score of 1* with inhibition zone; B = inhibition score of 1* without inhibition zone; C = inhibition score of 1; and D = inhibition score of 0.

branches developed symptoms of phytotoxicity in the form of brown discoloration on each side of the midrib and main veins. The injury was temporary, and these branches appeared normal the following year.

The bioassay data indicated that complete distribution is possible with root flare injection (Tables 1 and 3) but that the extra fungicide did not extend the period of effectiveness beyond that provided by the 3× rate. The average diameter of the trees that contributed to the estimates of distribution after 9, 10, and 12 mo (Table 3) was 78.5 cm (31 in.) or larger. Thus, complete distribution lasting through the second season is possible even in very large trees provided the dosage rate is adequate.

Treatments IX and X: MBC-P injected at three and six times the therapy label rate. The MBC-P dosage rate used for treatment IX (3×) was selected for evaluation because it is very close to what Kondo and Huntley (12) recommended for trees 61 cm in diameter. The dosage rate for treatment X (6×) is twice that of treatment IX.

Treatment X delivered 1.96 g of MBC-P per centimeter of diameter and resulted in complete distribution (Table 1). TBZ-P had to be injected at a rate of 3.73 g per centimeter of diameter (VI) before similar distribution and strength were achieved, suggesting that MBC-P was about twice as active per unit of active ingredient as TBZ-P. Although MBC-P was initially more active against *C. ulmi*, its activity in the newest radial wood disappeared after 13 mo (Table 1).

Treatments XI and XII: MBC-P injected at preventive and therapy label rates. The trees used for these treatments were bioassayed 14 days after injection rather than 20–29 days as with the other trees in the study. This should not have a significant effect on detection of the fungitoxicant if it is present in substantial quantities, but no activity was detected in any of the 116 samples taken from these trees (Table 1).

Uptake times. For trunk injections using relatively few holes and small volumes, the average time for five injections was 12.4 min (8–20 min) for the preventive rate and 21.5 min (19–25 min) for the therapy rate. The rate of 1.86 L/cm of diameter used to apply the 1×, 2×, and 3× TBZ-P treatments had an average time for 38 injections of 2 hr and 12 min and a range of 55 min to 5 hr. The rate of 2.23 L/cm of diameter used to apply the 3× and 6× MBC-P treatments had an average for nine injections of 2 hr and a range of 50 min to 3 hr. The rate of 2.98 L/cm of diameter used to apply the 4.8× TBZ-P treatment had an average time for 11 injections of 5 hr and 58 min and a range of 1 hr and 50 min to 24 hr.

TABLE 2. Treatment VII: Chemical distribution in American elms treated with thiabendazole hypophosphite at three times the maximum therapy label rate as a function of time since injection[a]

Trees sampled (no.)	Time (mo)	Distribution (%)	Distribution Range	Samples with * (%)[b]	Samples with IZ (%)[c]
4	>1	54.4	12–100	33.3	8.7
10	1	98.1	88–100	76.9	25.5
6	2	97.9	93–100	85.3	28.4
4	3	90.6	75–100	57.8	1.6
8	11	91.5	72–100	41.5	4.6
7	12	90.2	81–100	42.9	4.5
8	13	77.3	44–100	11.7	0.8
8	14	82.0	33–94	25.8	2.3
7	23	73.2	50–94	14.1	1.4
5	24	53.8	38–63	3.8	0
8	25	63.3	38–88	0.8	0
2	26	50.0	44–56	0	0
1	34	18.8		0	0
1	35	25.0		0	0
2	36	35.0	6–44	0	0
5	37	13.8	0–38	0	0

[a] Chemical concentration was 3.0 g/L and volume rate was 1.86 L/cm DBH.
[b] Complete inhibition of synnemata over entire cylindrical surface of disk.
[c] Inhibition zones on agar around disk.

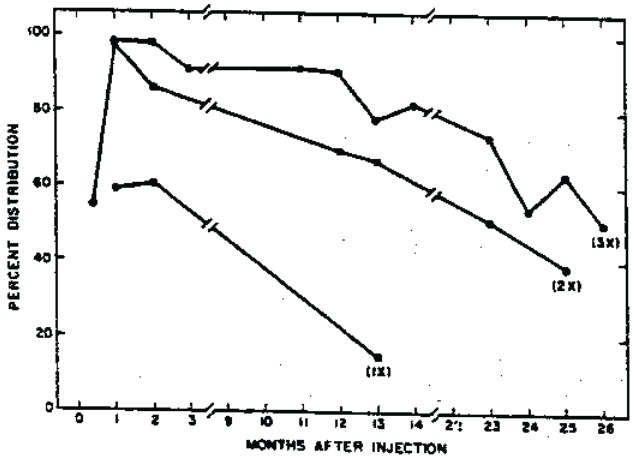

Fig. 3. Chemical distribution in American elms treated with TBZ-P at maximum therapy label rate (1×) and at two (2×) and three (3×) times maximum therapy label rate as function of time since injection.

DISCUSSION

Based on the bioassay data from the 2×, 3×, and 4.8× TBZ-P treatments and the 6× MBC-P treatment, complete distribution of the chemical in the periphery of the crown is possible with exposed-root-flare injection given adequate doses. There are, however, some inconsistencies in the bioassay data from treatments II and IV, the root-flare and trunk injections at the highest TBZ-P therapy label rate. One month after treatment, trunk injection resulted in better distribution of detectable chemical, and much stronger activity, than did root-flare injection. When trunk injected, the chemical apparently moved up the tree in bands, and where it was detectable the activity was comparatively strong because that part of the tree actually received more than its share of the fungicide. When the same dose was injected into exposed root flares with three to four times as many injection holes, distribution of detectable chemical decreased and there were no indications of strong chemical activity. More complete and even distribution probably accounted for this disparity in the activity of the fungicide.

Elliston and Walton (7) found that MBC-P at rates of 1.0 and 2.0 g/cm of diameter injected at ground level with injections spaced 15–23 cm apart resulted in nonuniform distribution within and between branches on any given tree. The dosage rates were the same as used in treatments IX and X, but we used more than three times as many injection holes and achieved very uniform distribution with the 2.0 g/cm rate (X). The disparity in

TABLE 3. Treatment XIII: Chemical distribution in American elms treated with thiabendazole hypophosphite at 4.8 times the maximum therapy label rate as a function of time since injection[a]

Trees sampled (no.)	Time (mo)	Distribution (%)	Distribution Range	Samples with * (%)[b]	Samples with IZ (%)[c]
12	>1	89.0	76–100	48.4	23.5
10	1	96.9	81–100	79.4	46.3
5	2	91.3	75–100	75.0	17.5
2	3	100.0		80.6	3.2
5	9	93.8	84–100	57.8	19.3
10	10	93.9	88–100	66.7	23.0
6	11	95.9	88–100	49.5	19.6
11	12	93.3	72–100	62.5	13.0
12	13	94.2	63–100	42.4	14.1
2	14	84.4	69–100	25.0	6.3
8	22	83.6	63–100	28.1	7.0
4	23	81.3	69–88	3.1	0
5	24	57.5	25–88	1.3	0
7	25	67.9	38–94	7.1	1.8
2	35	45.8	25–88	0	0

[a] Chemical concentration was 3.0 g/L and volume rate was 2.98 L/cm DBH.
[b] Complete inhibition of synnemata over entire cylindrical surface of disk.
[c] Inhibition zones on agar around disk.

distribution observed between root-flare and trunk injection suggests that the bioassay technique may not detect small amounts of chemical that might be sufficient to prevent infection by *C. ulmi*.

Foliar phytotoxicity observed on trees that were trunk injected or injected at ground level with TBZ-P dosage rates in excess of the maximum therapy label rate is additional indirect evidence for the inadequacy of trunk injections. When the 2× or 3× rate is injected in a manner that does not provide good distribution, the chemical is locally concentrated and parts of the tree are damaged, whereas other parts of the tree receive little or no chemical. These assumptions are further supported by the absence of phytotoxicity in elms properly root-flare injected with 3 and even 4.8 times the maximum amount recommended on the original label.

We believe that exposed-root-flare injections should use 6- to 8-mm-diameter holes spaced 10–20 cm apart, with enough root-flare surface area exposed to allow for about 0.89 injection sites per centimeter of stem diameter. Depending on the units of measure that are most convenient for the applicator, 0.89 sites per centimeter is equal to 1 site per 1.13 cm, or 2.26 sites per inch of diameter. For trees with a diameter of about 75 cm (30 in.) or less, this figure is appropriate under most circumstances. Accordingly, for root-flare injection, about one site for every 1.13 cm of stem diameter is advisable, and as much of the root flare and base of the tree should be exposed as necessary to accommodate the required number of injection sites. The results of this study indicate that complete and even distribution of the chemical will result.

The evidence gathered here and by others (10) clearly indicates that MBC-P has a significant effect on the DED fungus during the year of treatment, if properly applied in sufficient quantities. In agreement with Nishijima and Smalley (14) and Elliston and Walton (7), the data presented here indicate that the MBC-P label rates are completely inadequate. Kondo (10,11) has suggested that, for maximum distribution and effectiveness, systemic fungicide injections should use very low concentrations (0.25 g/L) and a minimum 24-hr injection time. Our data with the 6× MBC-P rate applied at 0.875 g/L indicate that complete distribution is possible with concentrations greater than 0.25 g/L and injection times of only 2 hr. Elliston and Walton (7) also reported 2- and 3-day uptake times for a volume rate equal to one half of what we used, but they used very few aboveground injection sites. Regardless of dosage rates, concentrations, uptake times, or the fact that carbendazim is significantly more active against the fungus per unit of active ingredient than thiabendazole (16), MBC-P did not move into new wood formed after injection, even when applied at a rate six times the maximum label rate. Nishijima (13) showed that MBC-P is temporarily very mobile within the tree, is quickly distributed to the foliage, and is lost as the leaves drop. In addition, much of the fungicide that does remain in the tree is unstable and degrades or is metabolized into nonfungitoxic by-products (14), or it may be deposited in localized areas and adsorbed by the cell walls (15). In any event, very little, if any, of the original fungitoxicant is present in the new radial wood formed after injection. Stillwell (21) has shown that 1–3 wk after injection little if any of the chemical was present in the outermost sapwood; after 7 wk, one of the trees in his study was diseased. Isolation and bioassay results indicated that the fungus was present in the new sapwood immediately adjacent and external to the wood containing the fungitoxicant. He concluded, as we did, that the lack of radial movement into new xylem formed after injection limits the potential usefulness of MBC-P.

In contrast to carbendazim, thiabendazole was present and could be detected in the newest radial wood 13, 25, and even 37 mo after injection. Distribution and relative strength of the activity were dependent on dose. These data confirm Nishijima's (13) conclusion that if initial concentrations of thiabendazole achieved in the crown are high enough, the chemical will move into new wood formed during seasons subsequent to the season of injection.

The original (1977) TBZ-P preventive label rate (0.931 g/cm) probably does not provide sufficient protection against DED, even though TBZ was detected in parts of the tree 1 mo after injection. One tree that was root-flare injected with this dosage rate became diseased shortly after it was injected and died the following year.

The maximum therapeutic label rate for TBZ-P (1.86 g/cm) is the only EPA-approved dosage rate for either chemical that significantly inhibits *C. ulmi* in the tree. The best detectable distribution achieved after 1 mo with root-flare injection of this dosage rate was 62.5%. These levels of chemical distribution are only those "observed" by means of bioassay; the possibility exists that there may be levels of chemical activity that cannot be detected by bioassay but that are high enough to prevent infection. Detectable distribution drops to very low levels 13 mo after injection, suggesting that significant protection does not extend into a second growing season. The dose equal to two times the highest TBZ-P therapy label rate (3.73 g/cm) was the lowest one that provided reasonably complete distribution of the fungicide 1 mo after injection. The triple therapy rates (5.59 g/cm) applied at 3.0 and 1.5 g/L, respectively, apparently did not perform equally in the tree. When diluted to 1.5 g/L, the chemical moved through the trees more slowly and never accumulated in the crowns to the same extent as when diluted to 2.0 or 3.0 g/L.

The TBZ-P rate equal to 4.8 times the highest rate on the old label was apparently safe for mature trees if well distributed, but the bioassay data were insufficient to suggest that the chemical was distributed more uniformly or moved into new wood for a longer period of time than with the triple therapy rate at 3.0 g/L. The average diameter of the trees used to estimate chemical distribution with the 4.8× rate after 9, 10, and 12 mo was 78 cm (31 in.) or greater (Table 3), suggesting that complete distribution in very large elms is possible with root-flare injection, even during the second season. However, the linear relationship between diameter and volume of functional sapwood may not hold for very small or very large elms. Elliston and Walton (7) observed higher concentrations of carbendazim in small trees than in large trees treated with the same dosage rate. Accordingly, we believe that the triple therapy rate may not always be enough for very large trees but that it can be too much for young trees.

Based on the evidence presented here, we believe that the triple therapy rate of TBZ-P root-flare injected at 3.0 g/L, 1.86 L/cm of diameter, is acceptably safe and is the most promising and cost-effective treatment of those evaluated. The fact that one treatment can provide nearly complete distribution of detectable chemical in new wood for two growing seasons, and provide enough chemical to be detectable in significant quantities into a third growing season, suggests that the cost of a second and perhaps third annual injection, as well as the damage from the second and third sets of holes, can be eliminated.

The amount and kind of injury resulting from systemic fungicide injections are important. Visible injury to the foliage can result from excess chemical or from uneven distribution of the correct dose. Our experience suggests that more fungitoxicant than necessary can be injected into living elms without causing visible injury if the chemical is uniformly distributed.

Phytotoxic injury also occurs at the injection sites. Recent work by Shigo and Campana (17) and Shigo et al (18) has shown that any kind of injection wound can lead to physiological problems for the tree, especially if repeated in successive years. Andrews et al (1) observed that discoloration of the wood occurs around and above any injection wound and that this discoloration is more extensive with TBZ-P applied at 3.0 g/L than with MBC-P applied at 0.875 g/L.

We believe that yearly injections to prevent Dutch elm disease will not be feasible, either economically or from the perspective of injection site injury, since proper injection requires so many injection holes. TBZ-P is the only commercially available chemical that will be present in the new wood during the second and third season after treatment, and then only if the total dose and the concentration of the injected solution are comparatively high.

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Techniques

A Qualitative Baiting Technique for Selective Isolation of *Rhizoctonia zeae* from Soil

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ABSTRACT

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A baiting technique was developed for selective isolation of *Rhizoctonia zeae* from naturally infested soil using fungicide-treated stem segments of cotton and a selective medium consisting of 2% water agar and benomyl, metalaxyl, penicillin G, and streptomycin sulfate at 10, 10, 50, and 50 μg a.i./ml, respectively. Cotton stem segments soaked in benomyl at 500 μg a.i./ml and metalaxyl at 100 μg a.i./ml or in benomyl at 1,000 μg a.i./ml were successfully used to isolate *R. zeae* from two naturally infested soils. Fungicide-treated stems were colonized in significantly higher numbers by *R. zeae* than untreated stems. The selective medium also increased recovery of *R. zeae* from colonized stems. Untreated stems were colonized by *R. solani*, binucleate *Rhizoctonia*-like fungi, *Pythium* spp., and a number of other common soil-inhabiting fungi.

Rhizoctonia zeae Voorhees is a soil-inhabiting fungus that was first isolated from diseased corn in 1934 (17). Since that time, *R. zeae* has been isolated and identified infrequently (5,6,14,16). It has been isolated with other *Rhizoctonia* spp. when a nonselective or semiselective isolation medium has been used.

Baiting techniques for studying the occurrence and distribution of *R. solani* Kühn have used seeds, paper disks, or excised plant parts as baits (2,3,8–10,13). These techniques are usually semiselective, and several *Rhizoctonia* spp. may be isolated. In this case, characterization of these fungi may be time-consuming because they are not readily distinguishable during initial growth in culture.

The objective of this study was to develop a baiting technique for the selective isolation of *R. zeae* from soil.

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