Distribution and Persistence of Methyl 2-Benzimidazole Carbamate Phosphate Injected into American Elms in Late Spring or Early Fall

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ABSTRACT

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Distribution and persistence of methyl 2-benzimidazole carbamate phosphate (MBC-PO_4) injected into American elms in late spring depended on rate of application but was independent of carrier volume over the ranges of application rates and volumes studied. The amount of MBC-PO_4 in leaves and twigs of trees injected in late spring decreased rapidly during the 3–4 mo after injection. Distribution and persistence were best with 6 and 12 times the recommeded rate for prophylaxis. The chemical was not detected in the spring following treatment. MBC-PO_4 injected into plantation elms in early fall persisted over winter and was well distributed in new growth the following spring.

Additional key words: Dutch elm disease, *Ceratocystis ulmi*, fungicide, chemical control.

Ceratocystis ulmi (Buism.) Moreau, the fungus that causes Dutch elm disease, spreads within infected American elm trees (*Ulmus americana* L.) primarily through vessels and other water-conducting elements of the current year's sapwood. Elms are most susceptible when the current year's spring vessels are near the surface of the sapwood and functioning as the primary conduits for water transport (May through early July in Connecticut).

Ideally, a fungicide for preventing Dutch elm disease should be toxic to *C. ulmi* at low concentrations, nonphytotoxic, mobile within and distributed evenly throughout the active water conducting system of the tree, and sufficiently persistent to remain at an effective concentration over the entire susceptible period of the elm. Salts of methyl 2-benzimidazole carbamate (MBC), an acid-soluble hydrolysis product of benomyl, have shown promise as agents for control of this disease (1,3,7–9). Application rates ranging from 0.08 to 2.8 g AI/cm dbh (diameter at breast height = trunk diameter 1.4 m above the ground) have been used in volumes of water ranging from 0.2 to 3.6 L/cm dbh (3–5,7,8). Typically trees are treated in late spring after the leaves have expanded and the spring vessels of the newest annual ring are functional. Late spring injection does not protect trees during the early part of the period of highest susceptibility.

The objectives of experiments reported here were to compare distribution and persistence of MBC injected at three application rates in late spring, to determine the effect of volume of carrier on distribution and persistence of MBC injected in late spring, and to determine the feasibility of protecting trees during the early part of the susceptible period by injecting them during the fall of the preceding year.

MATERIALS AND METHODS

Trees. American elms used in the experiments were growing at two locations: a college campus in Hartford, CT (campus elms), and at the Lockwood Farm in Hamden, CT (plantation elms). Campus elms ranged 10–97 cm dbh and 6–24 m in height; plantation elms were smaller and more uniform in size, ranging 15–23 cm dbh and 8–12 m in height.

Chemical. Lignasan BLP (0.7% methyl 2-benzimidazole carbamate phosphate) (MBC-PO_4) was supplied by E. I. duPont deNemours Co., Wilmington, DE 19801.

Injection technique. Trees were injected by using either the threaded pipe system recommended by the Elm Research Institute (ERI) (12) or a modification of the "nail-on" injector described by Gregory and Jones (6). An 11- to 15-L spray tank fitted with a 4-bar pressure gauge and an appropriate brass fitting for connecting it to the series of injectors was used with both systems.

The ERI injectors consisted of 1 cm diameter galvanized pipe nipples 10 cm long threaded at one end into a tee or elbow coupling. The free end of the pipe nipple was screwed into a hole drilled approximately 1.5 cm into the sapwood.

The "nail-on" injector which was based on the Gregory and Jones (6) design is illustrated in Fig. 1. It consists of a 1-cm diameter galvanized pipe nipple 11 cm long with the threads at one end extended to 3 cm and threaded into a 6.5 × 6.5 cm × 0.6-cm-thick steel plate predrilled to accept four 7.5-cm double-headed nails. The opposite end of the nipple is threaded into a tee or elbow coupling designed for use with flexible plastic tubing. Two to four gaskets cut from 0.6- to 0.8-cm-thick sheet neoprene with a 2.5-cm-diameter arch punch and centerpunched with a 1-cm-diameter cork borer were used between the steel plate and the smoothed bark surface to make a watertight seal.

Holes were drilled through the bark and into the outer two or three layers of sapwood at 15- to 23-cm intervals around the trunk as close to ground level as possible and preferably on root buttresses. A 0.9-cm diameter bit was used for ERI injectors, and a 1.2-cm diameter bit fitted with a 2.5-cm-diameter counterbore was used for the nail-on injectors. The latter device produces a hole in the sapwood that is larger in diameter than the injector pipe, allowing free access of the injected solution to the cut vessels in the outermost layers of wood.

When in place, the injectors were connected in series with the spray tank. Air was eliminated from the system by raising the pressure in the solution-filled tank and venting at the last injector in the series, which was fitted with an elbow or a vented coupling. The pressure in the system gradually was increased to 0.7 to 1 bar, with frequent checks for leaks in couplings and at injection sites. When all leaks had been eliminated, tank pressure was maintained between 0.7 and 1 bar for the duration of the procedure. If a tree required more than 15 L of solution, the pressure was released before the tank was empty, it was refilled, and the pressure was re-established.

When the injection was completed, the injectors were removed

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and washed in alcohol before reuse.

Sample collection, preparation, and bioassay. *Campus elms.* Branch samples containing at least 15 leaves were removed from distal portions of four major leaders per tree. As often as possible the leaders sampled were located in different quadrants of the tree canopy. Branches from which samples were taken were tagged at the first sampling date and resampled on the second date. Each sample was segregated into twig and leaf portions and frozen at −20 C until bioassayed.

Plantation elms. The same sampling procedure was followed except that the samples were taken from four sides of each tree, since most trees only had one leader.

Bioassays. Fifteen leaves per branch were chosen at random, and from each a 1-cm diameter disk, including the midrib, was punched from a point one third of the distance from the leaf tip to the petiole. Two or four twig sections 1.5 cm long × 1 to 5 mm diam (excluding bark) were sampled per branch. The bark was removed prior to bioassay.

Leaf disks were placed on 20 ml of Difco PDA in 10 cm square petri dishes seeded with 2×10^6 *Penicillium expansum* Raper and Thom spores per milliliter. Three leaf disks were plated (adaxial surface downward) on each plate and pressed into complete contact with the agar surface. For twig bioassays, plates containing 30 ml of *Penicillium*-seeded agar were used. Forceps were used to embed twig sections in the solidified agar. Two sections were bioassayed per plate. Plates were incubated at 20 C. Diameters of zones of inhibition were measured after the fungus began to sporulate, usually 48 hr after plating.

The *P. expansum* spores were harvested from cultures grown on Difco PDA for 5 days at 25 C. Spore suspensions were prepared in sterile deionized water containing 1 drop of Tween-80/100 ml and filtered through two layers of cheesecloth. Spore concentrations were determined with a hemacytometer and adjusted to 2×10^7 spores per milliliter. Aliquots (110 ml) of spore suspension were stored in plastic bottles at −20 C until use. Suspensions prepared and stored in this way remained viable for at least 6 mo, which allowed all bioassays for a given year to be made with spores from a single source.

Standard curves for estimating MBC-PO_4 contents of leaf and twig samples were prepared as follows: MBC-PO_4 prepared from analytical grade MBC by the method of Kondo (8) was used to prepare a stock solution containing 0.4 mg/ml in absolute methanol. Dilutions were made in reagent-grade ethyl acetate. For the leaf bioassays, 50 µl aliquots containing 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.60, 0.70, 0.80, 0.90, or 1.0 µg MBC-PO_4 were applied to each of three 1-cm diameter disks of Whatman No. 1 filter paper per concentration. Disks were air dried, plated on PDA seeded with *P. expansum,* and incubated as previously described. Inhibition zone diameters were measured, averaged for each concentration, and plotted against concentration to obtain a standard curve.

Three separate standard curves were prepared for twig samples 1–2, 2.1–4.0, and 4.1–6.0 mm in diameter, respectively. Twig samples 1.5 cm long from untreated trees were treated with 50-µl volumes of ethyl acetate solutions containing 0, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.4, 1.6, 1.8, or 2.0 µg MBC-PO_4 and air dried. Five twig samples of each size class were treated with each concentration and bioassayed as described for twig samples.

The content of MBC-PO_4 in ppm was estimated using the appropriate standard curve and representative tissue weights. Representative tissue weights were obtained by averaging weights of 50 random leaf disks and 10 twig samples of each size class. Thus, values reported are crude estimates of the MBC-PO_4 content of the tissue samples.

Effect of application rate on distribution and persistence. In mid-June, 1976, campus elms were divided into four groups of 13 to 15 trees and injected with MBC-PO_4 at rates of 0.16, 1, or 2 g AI/cm dbh using 0.8, 0.6, and 1.12 L of solution per centimeter of dbh, respectively. The fourth group of trees was left untreated. The 0.16 g AI/cm dbh rate approximates that recommended by the manufacturer for prophylactic treatment. Samples of current growth twigs and leaves were taken for bioassay approximately 2 and 12 wk after injection.

Effect of carrier volume on distribution and persistence. In late May and early June, 1977, the same three groups of campus elms treated in 1976 were injected with MBC-PO_4 at 1 g AI/cm dbh using volumes of 0.28, 0.56, or 1.12 L of solution per centimeter dbh. Prior to treatment, twig and leaf samples were taken from three representative trees in each group for bioassay to determine if MBC injected the previous year had persisted. Samples for bioassay were taken from all trees approximately 2 and 15 wk after injection.

Fall injection. Plantation elms were injected during the first week of October, 1976, with MBC-PO_4 at rates of 0.16, 1, or 2 g AI/cm dbh in 1.12 L of solution per centimeter dbh. Five trees were treated at each rate and five others were left untreated to serve as controls. Twig and leaf samples were taken for bioassay approximately 2 wk after treatment and in mid-June, 1977.

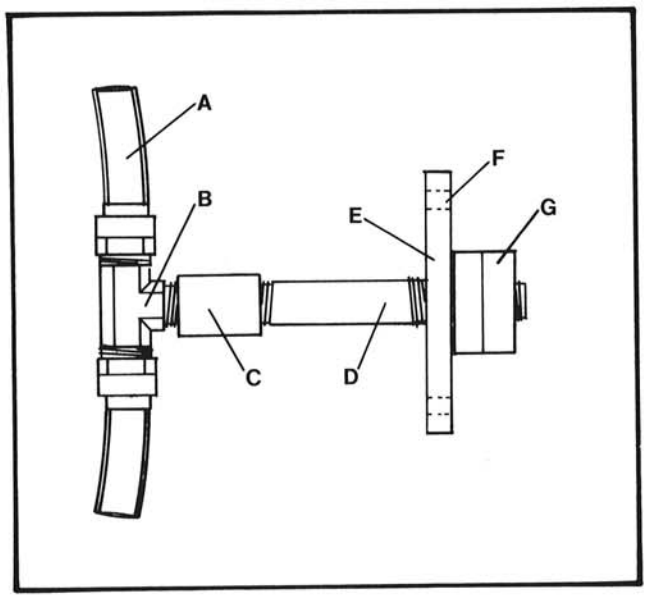

Fig. 1. Diagram of a nail-on tree injector used to inject elm trees with methyl 2-benzimidazole carbamate phosphate. Legend: **A,** flexible plastic tubing; **B,** brass tee fitting; **C,** galvanized 1-cm diameter pipe coupling; **D,** galvanized 1-cm diameter pipe nipple; **E,** steel plate; **F,** hole to accept nail; **G,** neoprene gaskets.

RESULTS

Effect of application rate on distribution and persistence. Bioassay results for campus elms treated at 0.16, 1, or 2 g MBC-PO_4 per centimeter dbh in late spring 1976 are summarized in Table 1. Bioassays of samples from untreated and treated trees before injection (not shown) were negative.

Trees treated at the lowest rate contained low or undetectable levels of MBC 2 wk after injection. Branch samples from only five of the 13 trees (39%) yielded twigs with positive bioassays. Samples from nine of those trees (69%) yielded leaves with positive bioassays. Distribution was uneven and MBC content was low for most trees in which the fungicide was detected. Four of the trees with positive leaf bioassays yielded no positive twig bioassays. Branch samples from three trees treated at this rate yielded no positive bioassays.

Twelve weeks after injection none of the twig samples and only 1% of the leaf samples from trees treated at the lowest rate contained detectable quantities of MBC-PO_4. The amounts detected were near the limit of detection of the method.

Bioassay results for trees treated at the two higher rates (1 and 2 g MBC-PO_4/cm dbh) were not significantly different from one another but were significantly different ($P = 0.01$) from results obtained with the lowest rate. Two weeks after injection, 97 and

98% of all branches sampled from trees treated at the 1- and 2-g rates, respectively, contained detectable quantities of MBC-PO_4. With few exceptions more than 70% of all leaves and twigs sampled from branches with positive bioassays contained detectable quantities of MBC-PO_4. The average quantity of MBC-PO_4 detected in a given tree ranged 1–54 ppm for leaf samples and 1–33 ppm for twigs. The highest average levels were detected in samples from the smaller trees (trees < 25 cm dbh). However, a consistent relationship between MBC content of leaf or twig samples and tree dbh was not observed.

Twelve weeks after injection the total number of branch samples with positive bioassays from trees treated at the 1- and 2-g rates had declined 20 and 17%, respectively (changes significant, $P = 0.05$). The decline in number of positive twig samples exceeded that for leaf samples (72 vs 23%, respectively, for the 1-g rate, and 57 vs 29%, respectively, for the 2-g rate). Highly significant decreases in MBC-PO_4 content of leaf and twig samples also occurred ($P = 0.01$) (83 and 90%, respectively, for the 1-g rate, and 80 and 90%, respectively, for the 2-g rate).

Effect of carrier volume on distribution and persistence. Bioassays of samples taken from untreated and treated trees before injection were negative. Volumes calculated for the two lower rates were injected successfully. Volumes calculated for the 1.12-L rate were taken up by only three of the 14 trees assigned to that treatment. On average, 72% of the calculated volume was injected into these trees.

No significant differences in percentages of branch, leaf, and twig samples with positive bioassays or in average MBC content of leaf and twig samples were found for the three rates (Table 2). For samples taken 2 wk after injection, treatment means ranged 92–98% for branch samples with positive bioassays, 65–77% for leaf samples, and 74–91% for twig samples. Average MBC-P0$_4$ content ranged 7–12 ppm for leaves and 5–8 ppm for twigs.

For all treatments the percentages of branch, leaf, and twig samples with positive bioassays and MBC-PO_4 contents of leaf and twig samples were significantly lower ($P = 0.05$) 15 wk after injection. Treatment means for percent branch, leaf, and twig samples with positive bioassays declined 27–46%, 29–70%, and 78–92%, respectively. Treatment means for MBC-PO_4 content of leaf and twig samples declined 83–94% and 85–98%, respectively.

Fall injection. Bioassays of samples taken in October from untreated trees were negative.

Of the branch samples taken 2 wk after injection from trees treated at 0.16-, 1-, and 2-g rates, 35, 65, and 100%, respectively, yielded positive bioassays (Table 3). A larger proportion of twig than leaf samples were positive for all rates. For trees treated at the 0.16-g rate, average MBC-PO_4 content was near the limit of detection of the method. With the exception of leaf samples from trees treated at the 1-g rate, average concentrations detected in samples from trees treated at the two higher rates were significantly greater ($P = 0.05$). The amounts of MBC-PO_4 in leaves and twigs sampled from trees treated at the two higher rates were not significantly different.

The proportion of branch samples with positive bioassays was higher in June than the previous October. Sixty-five percent of branches sampled from trees treated at the 0.16-g rate and 100% of branches sampled from trees treated at the two higher rates yielded subsamples with positive bioassays. The concentrations of MBC-

TABLE 1. Means of bioassays for methyl 2-benzimidazole carbamate phosphate (MBC-PO_4) in campus elms sampled 2 and 12 wk following injection 8–17 June 1976

Injection rate of MBC-PO_4 (g/cm dbh)[a]	Postinjection 2 wk					Postinjection 12 wk				
	Branch samples positive[b]	Subsamples positive (%)		Average MBC-PO_4 content (ppm)		Branch samples positive[b]	Subsamples positive (%)		Average MBC-PO_4 content (ppm)	
		Leaf	Twig	Leaf	Twig		Leaf	Twig	Leaf	Twig
0.16	1.8	46	38	1	1.7	0.3	1	0	0	0
1.0	3.9	83	94	18.3	10.2	3.1	60	22	3.2	0.5
2.0	3.9	79	96	21.7	11.6	3.2	50	39	4.4	1.2

[a] dbh = trunk diameter measured at breast height.
[b] Four branch samples, one from each canopy quadrant, were taken from each tree on 28 June and 9 September 1976.

TABLE 2. Means of bioassays for methyl 2-benzimidazole carbamate phosphate (MBC-PO_4) in campus elms sampled 2 and 12 wk following injection on 27 May–8 June 1977 with 1 g MBC-PO_4/cm trunk diameter

Injected volume (L/cm dbh)[a]	Postinjection 2 wk					Postinjection 12 wk				
	Branch samples positive[b]	Subsamples positive (%)		Average MBC-PO_4 content (ppm)		Branch samples positive[b]	Subsamples positive (%)		Average MBC-PO_4 content (ppm)	
		Leaf	Twig	Leaf	Twig		Leaf	Twig	Leaf	Twig
0.28	3.9	65	85	10	6	2.1	46	19	1.7	0.9
0.56	3.9	77	91	12	8	2.4	23	16	2	0.3
1.12	3.7	73	74	7	5	2.7	29	6	0.4	0.1

[a] dbh = trunk diameter measured at breast height.
[b] Four branch samples, one from each canopy quadrant, were taken from each tree on 22 June and 14 September 1977.

TABLE 3. Means of bioassays for methyl 2-benzimidazole carbamate phosphate (MBC-PO_4) in plantation elms injected on 5–8 October 1976 with 0.16, 1, or 2 g MBC-PO_4/cm of trunk diameter at breast height

Injection rate of MBC-PO_4 (g/cm dbh)[a]	19 October 1976					20 June 1977				
	Branch samples positive[b]	Subsamples positive (%)		Average MBC-PO_4 content (ppm)		Branch samples positive[b]	Subsamples positive (%)		Average MBC-PO_4 content (ppm)	
		Leaf	Twig	Leaf	Twig		Leaf	Twig	Leaf	Twig
0.16	1.4	1.6	30	0	0.4	2.6	23.6	26.8	0.4	<1
1.0	2.6	41.8	60	2.6	5	4	90	95.2	11	8
2.0	4	80.2	92.4	5.2	10.4	4	99	88.4	23.6	10

[a] dbh = trunk diameter measured at breast height.
[b] Four branch samples, one from each canopy quadrant, were taken from each tree on each date.

PO_4 detected in trees treated at the 0.16-g rate remained near the limit of detection of the bioassay. Concentrations in samples from trees treated at the two higher rates were significantly higher ($P = 0.05$) than in trees treated at the low rate. The contents of MBC-PO_4 in twigs from trees treated at the two higher rates were not significantly different. However, leaf samples from trees treated at the 2-g rate contained significantly more MBC-PO_4 than leaves treated at the 1-g rate ($P = 0.05$). Likewise, leaf samples taken in June from trees treated at the two higher rates contained significantly more MBC-PO_4 than leaves sampled the previous October ($P = 0.01$). The contents of MBC-PO_4 in leaves from trees treated at the 0.16-g rate were not significantly different in October and June. The amount of MBC-PO_4 in twigs sampled on the two dates were not significantly different for any of the rates tested.

DISCUSSION

If the proportion of branch samples yielding at least one subsample with a positive bioassay is considered, and if it is valid to extrapolate from results from four widely separated branches to the situation for an entire tree, the MBC-PO_4 injected as described was well distributed within the branch system of American elms. This was most apparent from data for samples taken 2 wk after treatment at rates of 1 or 2 g/cm dbh. Although 2 wk after treatment MBC-PO_4 could be detected in nearly all branches of trees treated at these rates, the uniformity of distribution within a branch, between branches on a given tree, and between trees within treatments was highly variable. This is evident from the average estimated MBC-PO_4 contents of leaf samples and the proportions of samples yielding positive bioassays, both of which varied widely for trees within a given treatment. This variation in content and distribution was probably due to a combination of factors: (i) The water status of the tree at time of injection, which could have affected the proportion of the injected material that moved downward into the roots and was released into the soil. (ii) The size of the tree. Dosage is customarily calculated on the basis of tree diameter, whereas the total volume of the current season sapwood is more directly related to the square of trunk diameter. Thus, small trees received larger doses per unit of sapwood volume than did larger trees. The degree of complexity of the vascular system also is related to tree size. Mature American elms typically have numerous leaders ascending from the trunk, whereas small trees have one or two main branch systems. (iii) The geometry of the vascular system at points of injection and the number and spacing of points of injection are likely to influence distribution. The number of injectors could have had an important effect on distribution since they were arranged in series. When numerous injectors are used in series, a pressure drop occurs between the first and last injector in the series, leading to injection of less material at the end of the series than at the beginning. In addition, differences undoubtedly occurred in uptake through different holes around a tree, depending upon the particular set of vessels intercepted, the water status of the parts of the tree they served, and the extent to which they were plugged during or after drilling.

The most easily predicted trend was the change in MBC-PO_4 content with tree diameter—one would expect samples from large trees to contain, on the average, less MBC than samples from small trees. Although this trend is suggested in data for samples taken 2 wk after injection of campus elms with 1 and 2 g MBC/cm dbh it was less evident in data from the carrier volume experiment conducted with the same trees 1 yr later. The explanation for the difference in results for the two years probably lies in the complications noted above.

The fungicide was difficult to detect in trees treated at the label rate for prophylaxis; this confirmed Nishijima's observations (10). If it can be assumed that the limit of detection of the bioassay approximates the limit of inhibitory activity of MBC to *C. ulmi* in the tree, the application rate recommended for prophylaxis (approximately 0.16 g/cm dbh) should be inadequate to protect these elms from Dutch elm disease. The reported LD_{50} of MBC for mycelial growth is 3 ppm (11). The limit of detection of the bioassay with *P. expansum* is 1–2 ppm. *Ceratocystis ulmi* is less sensitive than *P. expansum* to MBC-PO_4. (J. E. Elliston, *unpublished*).

As indicated by bioassay results, trees treated in late spring at the higher rates should be much better protected, especially during the weeks immediately following injection. Levels of MBC-PO_4 declined rapidly over the 12-wk period after treatment. Samples taken from these trees the following spring did not contain detectable quantities of MBC. This supports conclusions from other studies (2) that, for continuing protection, MBC injections for prevention of Dutch elm disease must be repeated annually.

The lack of a significant difference between MBC-PO_4 contents of samples from trees treated at the 1- and 2-g rates was unexpected and cannot be explained.

Over the range of volumes studied (0.28–1.12 L/cm dbh), volume of carrier had no significant effect on distribution. This, also, was contrary to expectations. If higher carrier volumes had provided improved distribution, the longer time required to inject the trees would have made the procedure economically impractical. Few of the trees treated in 1977 at the highest volume rate took up the calculated dose after as many as three days. With the second highest volume rate, many trees required 2 days for completion of the treatment.

The rates of uptake in spring 1977 were lower than those for the same trees in the spring of 1976. Most trees treated at the 2-g rate in 1976 (1.12 L/cm dbh) took up the complete dose in 1–3 days. Injections in 1977 were made about 2 wk earlier than in 1976. Either the water deficits in the trees were much greater at the time of injection in 1976 than in 1977 or the functional portion of the vascular system was less mature and had a lower total volume when injected in 1977.

Injection of MBC-PO_4 into elms in late spring cannot protect them from infection during the earliest part of the growing season, a period when they are highly susceptible. Injection in early fall of the previous year, a few weeks before leaf drop, shows promise for protecting elms throughout the highly susceptible period of their annual growth cycle. Trees injected in early October, 1976, retained MBC-PO_4 overwinter. The following spring the material moved into the new growth with distribution more complete than the previous fall. The MBC-PO_4 that is injected into the sapwood of an elm one year is not trapped in that wood, but can be transported into wood produced the next year. Since amounts detected in trees treated at the 1- and 2-g rates in mid-June were comparable to those detected in trees 2 wk after late spring injection, it is likely that the fall treatment provided protection equivalent to that provided by treatment in late spring but, in addition, protected the trees during the early part of the growing season. The persistence of MBC-PO_4 in fall-injected trees was not determined.

The fate of MBC-PO_4 injected into campus and plantation elms was not followed and remains uncertain. The quantities detected in leaves and twigs decreased rapidly during the 3–4 mo period following late spring injection. Samples taken during the following spring did not contain detectable amounts of MBC-PO_4. The chemical injected in late spring was not stored in the sapwood or root tissue over the winter, otherwise it should have been detectable in new growth the following year. Undoubtedly much of the material is lost from the trees when the leaves drop in autumn; it is water soluble and would be expected to be transported from the sapwood into the leaves with the transpiration stream. This suggestion is supported by results of the fall injection experiment. This cannot be the complete answer, however, since leaf MBC-PO_4 content decreased in the period between 2 and 12–15 wk after late spring injection. The material could conceivably have been transported irreversibly to tissues deep within the tree, become bound to cell walls or other host components hindering diffusion and detection by bioassay, it could have undergone enzymatic or spontaneous decomposition, leaching by rain, or any combination of these.

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