Root Uptake of Soil-Injected Benomyl and Its Detection within *Ulmus americana*

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Roots, stems, and leaves of greenhouse and nursery elms were bioassayed for benomyl content after the soil around the trees had been injected with varying concentrations of the fungicide. Benomyl, or a fungistatic derivative, was detected in all tissues of greenhouse elms within days following benomyl injection. Benomyl content of elm tissues is reported as the concentration of a chloroform (2.5 ml/g fresh weight) extract of the tissue as determined by bioassay. The micrograms per milliliter (parts per million) of benomyl detected in the roots and stems were approximately equal. At high rates of benomyl application, the accumulation in leaves was greater than 50 μg/ml while in roots as high as 20.2 μg/ml was detected. In field-grown elms the concentration of benomyl was highest during the year of application and substantially lower in succeeding years. At a sufficiently high rate of application, benomyl was detected in leaves and roots 4 years after treatment at a level that inhibited the growth of *Ceratocystis ulmi*, the causal agent of Dutch elm disease.

When the systemic fungicide benomyl (methyl 1-(butylcarbamoyl) benzimidazol-2-yl carbamate) is injected into soil as a concentrated aqueous suspension, it controls Dutch elm disease, fungus causal agent *Ceratocystis ulmi* (Buism.) C. Moreau, in *Ulmus americana* (American elm) for several years following application (Biehn, 1973; Neely, 1973). A concerted effort to determine the level of benomyl or its derivative MBC (methyl 2-benzimidazolecarbamate) in elm tissues, that is sufficiently high to prevent Dutch elm disease, has not been made. Earlier determinations were made by Biehn (1973).

The purpose of this study was to determine the quantity of benomyl, or a fungitoxic breakdown product, in elm tissues at varying times following soil application of the fungicide. Our method of bioassay used chloroform as the solvent to extract benomyl MBC from plant tissues. Similar methods of bioassay have been employed by other researchers to detect benomyl (Erwin, 1969; Gregory et al., 1971).

EXPERIMENTAL SECTION

Materials. The American elms used in field tests were 8–10 m tall in rows 2 m apart at the Illinois Natural History Survey arboretum near Urbana. Elms treated in 1970, 1971, and 1972 were spaced 1 m apart, and those treated in 1973 and 1974 were 2 m apart. Benomyl was injected into the soil around the test trees with a 189.2-l. hydraulic power sprayer (John Bean Division-FMC Corporation, Lansing, Mich.) which forced the aqueous benomyl suspension through a root-feeding needle at 14 kg/cm^2. The injection sites were 75 cm apart and 45 cm deep and were at least 50 cm from the base of any tree. At each site 3.8 l. was injected. Each treatment block contained 12 trees. Elms treated in 1971, 1972, and 1973 and one block of elms treated in 1974 were treated at the rate of 36 g (active ingredient) of benomyl per m^2 of soil surface. The elms treated in 1970 and one block of elms treated in 1974 received 18 g of benomyl/m^2. Another block of elms treated in 1974 received 9 g of benomyl/m^2. All soil injections were applied between April 30 and May 31.

Greenhouse trials involved 3-year-old seedlings 0.5 to 1.5 m tall, each planted in 3.4 l. of a potting mixture composed of sand, peat, and soil in a 1:1:1 ratio. Benomyl was injected into the soil of the cans with a hand-held 10-ml syringe with a 9-cm long hypodermic needle. The desired weight of benomyl was suspended in 100 ml of water and 10 injections were made of 10 ml each at random sites within the can.

Bioassay. A chloroform-extraction method was used to bioassay leaves, stems, and roots. Leaves for assay were selected at random and divided into 2-g (fresh weight) samples, shredded by hand, placed in a mortar with 5 ml of chloroform, and ground with a pestle for 2 min; the solid remains were discarded. Stems and roots were cut into 1-cm long pieces, divided into 2-g (fresh weight) samples, and immersed in 5 ml of chloroform for 30 min. Paper assay disks (12.7 mm in diameter) were dipped into the chloroform extraction and placed in petri dishes containing 10 ml of potato dextrose agar. After 1 h each plate was sprayed with a conidial suspension (ca. 5×10^4 conidia) of one assay fungus. Three fungi were used in the assays: *C. ulmi*, *Verticillium albo-atrum* Reinke and Berth., and *Penicillium expansum* L. Plates were incubated for 48 h at 23 °C before zones of inhibition, from disk edge to fungal mycelium, were measured.

RESULTS AND DISCUSSION

Preliminary studies using standard concentrations of benomyl dissolved in chloroform indicated a curvilinear relationship between micrograms per milliliter of benomyl and size of the zone of inhibition. Earlier studies also indicated that the chloroform-extraction assay detected the presence of benomyl at concentrations as low as 0.1 μg/ml and gave exact quantitative results at concentrations above that minimum (Figure 1). *P. expansum* was found to be more sensitive to benomyl than *C. ulmi* or *V. albo-atrum*.

In both greenhouse and field tests the uptake of benomyl was rapid. In greenhouse elms levels of up to 2.4 μg/ml benomyl were detected after only 7 days (Table I). Maximum accumulation in roots and stems was 3.0 μg/ml, occurring after 14 days; maximum accumulation in the leaves was at least 38.5 μg/ml occurring after 14–21 days. The amount of benomyl bioassayed from leaf samples was often 10 times larger than those from woody plant parts. Field results confirmed greenhouse results when the equivalent rate of 36 g of benomyl/m^2 was applied. At this rate the largest accumulation also occurred in the leaves, as indicated by an inhibition zone of 12.8 mm or 5.5 μg/ml (Table II). In the field, the maximum accumulation within both roots and leaves was detected after 60 days, as

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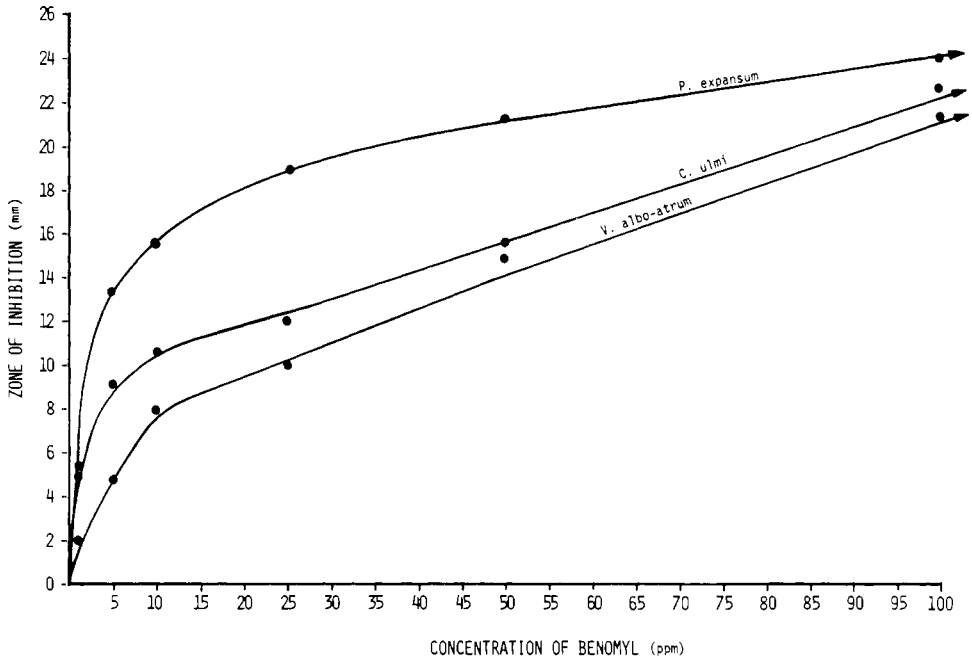

Figure 1. Different amounts of benomyl dissolved in chloroform resulted in concentrations of 0.01, 0.1, 1.0, 5.0, 25.0, and 50.0 μg/ml (ppm). When absorbed by paper disks and placed on potato dextrose agar seeded with benomyl-sensitive fungi and incubated for 48 h at 23 °C, varying zones of inhibition resulted. Seventy-five measurements were made at each concentration.

Table I. Influence of Time following the Injection of Benomyl into the Soil at the Rate of 36 g (Active Ingredient)/m² upon the Amount of Benomyl Detected in Assays of Chloroform-Extracted Benomyl from Greenhouse-Grown Elms

Days following benomyl application	μg/ml of benomyl (ppm)								
	Roots			Stems			Leaves		
	P. e.[a]	C. u.[a]	V. a-a.[a]	P. e.	C. u.	V. a-a.	P. e.	C. u.	V. a-a.
7	1.2[b]	1.2	0.8	1.1	0.5	1.4	1.9	1.7	2.4
14	2.0	1.9	3.0	2.4	2.1	0.3	>50.0	31.0	38.5
21	1.1	1.5	1.9	1.9	1.1	2.4	>50.0	49.0	>50.0
28	0.7	1.0	0.8	1.1	1.1	1.6	>50.0	>50.0	>50.0
35	1.1	0.4	0.8	2.4	1.4	5.5	10.8	19.2	25.7
Untreated	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Assay fungi used were *Penicillium expansum*, *Ceratocystis ulmi*, and *Verticillium albo-atrum*. [b] Each value represents the average of 30 measurements.

Table II. Influence of Rate and Time of Application upon the Amount of Benomyl Detected in Nursery Elms[a]

Rate of application, g/m²	Month of assay	μg/ml (ppm) of benomyl					
		Leaves			Roots		
		P. e.[b]	C. u.[b]	V. a-a.[b]	P. e.	C. u.	V. a-a.
36	July	0.7[c]	0.7	4.5	1.5	1.1	1.6
36	Aug	5.5	2.7	0.8	1.1	0.5	3.0
36	Sept	4.6	2.5	0.9	1.8	-[d]	4.8
18	July	1.1	-	0.4	0.1	-	1.0
18	Aug	0.3	-	0.6	0.3	0.4	0.3
18	Sept	-	-	0.3	-	-	0.1
9	July	-	-	0.5	0.3	0.3	0.4
9	Aug	0.1	-	0.4	-	-	0.3
9	Sept	-	-	0.3	-	-	-
	Untreated	0.0	0.0	0.0	0.0	0.0	0.0

[a] Field plots of elms were soil injected with benomyl at 36, 18, and 9 g (active ingredient)/m² on May 29, 1974. [b] The assay fungi used were *Penicillium expansum*, *Ceratocystis ulmi*, and *Verticillium albo-atrum*. [c] Each value represents the average of 75 measurements. [d] A dash indicates a detectable value less than 0.1 ppm.

Table III. Influence of Time following Treatment upon the Amount of Benomyl Detected in Nursery-Sized American Elms (Persistence)

Year of benomyl application[a]	μg/ml (ppm) of benomyl					
	Leaves			Roots		
	P. e.[b]	C. u.[b]	V. a-a.[b]	P. e.	C. u.	V. a-a.
1974	5.4[c]	2.6	3.2	1.1	0.4	0.8
1973	0.2	0.3	1.3	0.3	-[d]	1.0
1972	-	-	-	0.3	-	0.4
1971	-	-	-	0.2	0.4	0.9
1970	0.0	0.0	0.0	-	-	0.7
Untreated	0.0	0.0	0.0	0.0	0.0	0.0

[a] All soil injection done between April 30 and May 31 of the respective years at the rate of 36 g (active ingredient)/m² for 1971–1974 and 18 g (active ingredient)/m² in 1970. Assayed Sept 1974. [b] Assay fungi used were *Penicillium expansum*, *Ceratocystis ulmi*, and *Verticillium albo-atrum*. [c] Each value represents the average of 75 measurements. [d] A dash indicates a detectable value less than 0.1 ppm.

compared to 14 and 14–21 days in the greenhouse.

The amount of benomyl applied had a significant effect upon the amount accumulated by the elms. Field data indicate that a reduction by 75% in rate of application resulted in a decrease of accumulated benomyl in leaves from 4.6 μg/ml at 36 g/m² to <0.1 μg/ml at 9 g/m² (Table II). Similar trends in accumulation were noted in root assays.

Benomyl was persistent in the soil and was detected in all elms treated from 1970 to 1974. The amount of benomyl in roots was greater than the amount detected in leaves for every year except 1974, although 1973 treatments were similar (Table III). The amount of benomyl detected in the roots was greatest the year of application. Thereafter, a fairly constant amount of accumulation was detected, averaging 0.4 μg/ml. Leaf assays made more than 1 year following treatment indicated accumulated levels of benomyl not significantly different from untreated trees. Chloroform-extraction procedures detected benomyl for five growing seasons in root samples and for at least two growing seasons in leaf samples.

Although the side effects of the injection of benomyl into the soil are unknown, literature is available which states that the practice is effective in disease control (Smalley, 1971; Stipes, 1973). At the rate of application required for efficacy, benomyl or a benomyl derivative can be detected by bioassay in leaves, wood, and bark of treated elms (Biehn and Dimond, 1971; Hock et al., 1970; Zaronsky and Stipes, 1969). Inhibition of spore germination on seeded agar plates may not be sufficiently sensitive to detect the fungicide (Smalley, 1971; Hock and Schreiber, 1971), the principal limitation of the seeded plate bioassay being the sensitivity of the test fungus. More sensitive bioassay methods have been used successfully (Smalley et al., 1973). These bioassays will detect benomyl at concentrations as low as 0.01 μg/ml (Black, 1975).

The amount of benomyl required for efficacy against Dutch elm disease has not been established. In in vitro tests a concentration of 0.5 μg/ml of benomyl stopped growth of *C. ulmi*, while at 0.1 μg/ml growth was only slightly inhibited (Edgington et al., 1971; Hart, 1972). Neely (1973) noted, using the same field trees as in this test, that the 36 g/m^2 rate resulted in complete control of Dutch elm disease, while the 9 g/m^2 rate was only partially successful. His results, combined with this study, would show that detected levels of accumulation of not less than 0.5 μg/ml in the leaves would indicate sufficient benomyl uptake for total Dutch elm disease control. Levels of accumulation less than 0.5 μg/ml were not consistently successful in preventing *C. ulmi* colonization.

Sufficient benomyl accumulation to afford protection is rapidly reached. In the greenhouse levels of accumulation in the leaves of 0.5 μg/ml were reached only 7 days after treatment. In field tests, such levels were reached within 30 days following treatment, at the same rate.

The soil injection of benomyl will result in a detectable systematic accumulation of benomyl within the elm. Benomyl, so applied, will remain available to the tree in amounts that can control Dutch elm disease beyond the year of application. Levels of accumulation of benomyl as low as 0.1 μg/ml can be readily detected using the simple, inexpensive chloroform-extraction assay of leaves outlined herein. Such an assay, combined with the proper assay fungus, allows for a sensitive assay of whole-tree benomyl content, using only a small amount of tissue without disturbing the landscape.

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Toxicity, Penetration, and Metabolism of Chlordimeform and Its *N*-Demethyl Metabolite in Cabbage Looper Larvae

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The insecticide chlordimeform and its *N*-demethyl metabolite, *N'*-(4-chloro-*o*-tolyl)-*N*-methylformamidine or demethylchlordimeform, were more toxic to third instar cabbage looper larvae than to fifth instar larvae, with the parent formamidine being the more toxic in each case. When both larval instars were treated topically with the same amount of chlordimeform-^{14}C, penetration was slower in third instar larvae than in fifth instar larvae. There were no apparent qualitative differences in the chlordimeform metabolites between the two larval instars but quantitative differences were evident. Third instar larvae converted chlordimeform to demethylchlordimeform and to polar metabolites slower than did fifth instar larvae. Metabolism studies with demethylchlordimeform-^{14}C revealed a similar trend between the two instars. The slower penetration and metabolism of chlordimeform in third instar cabbage looper larvae as compared to fifth instar larvae may explain, at least in part, the differential toxicity.

Chlordimeform has an interesting spectrum of insecticidal activity, being toxic primarily to eggs and early instar larvae of some lepidoptera. For example, with the cabbage looper, *Trichoplusia ni* (Hübner), an economically important pest, susceptibility to chlordimeform decreases with increasing larval instars (Kuhr, 1974), and the basis for this phenomenon is obscure. In many instances selective toxicity can be explained on the basis of differences

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