Distribution of Benzimidazole Fungicides Following Pressure Injection of Pear Trees at Several Growth Stages

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ABSTRACT

Pressure injection of solubilized thiabendazole (TBZ) and methyl 2-benzimidazolecarbamate (MBC) into the trunk of pear trees, shortly before leaf fall, resulted in rapid translocation of the chemicals to the branches and leaves. During the next growing season the fungicides were further translocated into the new growth and gradually accumulated in the leaves. Injection during dormancy resulted in poor primary and limited secondary distribution. When the fungicides were injected during spring, considerable primary distribution was evident. However, there was a lag period before the chemicals started to accumulate in the emerging leaves. Observations for scab infection on leaves, caused by *Venturia pirina,* revealed that the disease was confined to the untreated trees.

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Additional key words: systemic fungicides, pear scab.

Pressure injection of fungicides into shade and fruit trees to control vascular diseases has recently become attractive due to the development of various systemic fungicides as well as several injection techniques (1, 4, 5, 6, 7, 8). Reduction of mycoplasma disease severity by pressure injection of tetracycline was demonstrated (2, 6, 9). Gregory et al. (3) reported that benomyl could be injected into the trunk of shade trees, and in oak trees some subsequent translocation of the fungicide to the crown was evident. We have shown (8) that when thiabendazole (TBZ) is injected under pressure into apple trees, a substantial amount is translocated into the branches and leaves. We distinguished between two phases in the distribution process: (i) Primary distribution, which is an immediate consequence of pressure injection, and (ii) secondary distribution, in which the fungicide is translocated in the plant via the transpiration stream. However, the potential of this method has not been sufficiently explored. The purpose of this investigation was to study the distribution pattern of benzimidazoles in pear trees at various stages of growth, and the accumulation of the fungicides within the leaves as a potential means for controlling foliar diseases. A preliminary report of this work has been published (10).

MATERIALS AND METHODS.–TBZ (Merck and Co. Inc., Rahway, N.J.) and methyl 2-benzimidazolecarbamate (MBC) (BASF AG, Ludwigshaven, Germany), both technical grade, were used. To facilitate injection, TBZ was solubilized in hypophosphorous acid as described elsewhere (8). MBC was dissolved in 1.0 N hydrochloric acid, 30 g/liter, yielding a final pH of 0.4.

Sampling and assaying of fungicides in branches and leaves were performed as described in a previous work (8). MBC and TBZ were bioassayed with *Penicillium* sp. and *Verticillium dahliae* Kleb., respectively.

Experiment 1.–Pear trees (*Pyrus communis* L. 'Spadona'), 5 yr old, were selected for this trial. The trees, grown on trellises, had branches up to 4 m long. The injection procedure was as follows: TBZ or MBC stock solutions were diluted with water to a concentration of 10 or 2 g/liter, respectively, and placed in 10-liter knapsack sprayers pressurized with CO_2 (2.026×10^5 pascals). Two pieces of latex tubing [I.D. 6.3 mm (0.25 in), 100 cm long] were attached to the boom of the sprayer. An expandable plastic device, normally used for insertion of screws into plaster walls, was forced into the open end of each latex tube. Two holes, 11 mm in diam and 60 mm deep, perpendicular to each other, but in different planes, were drilled into the trunk ca. 25 cm above soil level. The tubes ends containing the plastic devices were tapped into the bore holes. One liter of solution was injected under pressure from the sprayer into each of the treated trees, the amount being determined by weighing the sprayer during injection. The fungicides were injected either in the autumn (22 November 1972) close to leaf fall, or in the spring (5 March 1973) at. bud burst. Each treatment was applied to a single tree with five randomized replicates.

Experiment 2.–Ten-yr-old pear trees, cultivar Spadona, pruned to four limbs, were injected either in dormancy (14 Feb 1973), or 3 wk after bud burst (29 Mar 1973). Amounts of 1.25 or 5.00 g MBC in 166 ml of solution were injected. To obtain the lower dosage, the stock solution was diluted with hydrochloric acid of a similar pH. The solutions were injected into the limbs 1 m above ground as described elsewhere (8).

RESULTS.–*Experiment 1.* –Trees injected in autumn were examined 7 and 21 days after treatment. At the first sampling date, all trees already were in the stage of advanced shedding. The translocation of fungicides in the trees was deduced

TABLE 1. Content of fungicides in leaves of pear trees injected in autumn

Time after injection (days)	Methyl 2-benzimidazolecarbamate		Thiabendazole	
	Green leaves[a] (μg/g)	Necrotic[b] leaves (μg/g)	Green leaves (μg/g)	Necrotic leaves (μg/g)
7	20[c]	>230	29	423
21	57	—	52	—

[a]All leaf samples were collected from a height of 2 m from the soil level.

[b]Necrosis due to phytotoxicity.

[c]Fungicide content is expressed as μg/g fresh wt.

from their accumulation in the leaves. Phytotoxic symptoms, from marginal to complete necrosis, were evident on some leaves in trees treated with both compounds. Extremely large quantities of TBZ and MBC were determined in the necrotic leaves as compared with the unaffected ones (Table 1). Twenty-one days after treatment, the few green leaves that still remained contained more fungicide than in the 7-days sampling. The following spring, at green tip stage (5 Mar 1973), traces of MBC were detected in the opening buds (1.3 μg/g fresh wt). TBZ-treated trees were not assayed.

Five weeks after bud burst (9 Apr), leaves from new terminal (extension) shoots at ca. 2 m height in both autumn- and spring-injected trees, were sampled according to their age: basal (oldest), apical (youngest) and intermediate leaves. The content of fungicide was highly dependent upon leaf age; older leaves accumulated much more of the toxicant (Table 2). In autumn-treated trees, both compounds were present in the leaves at a higher concn than in leaves from trees injected in spring. At the fully developed foliage stage, 11 wk after bud burst (20 May), leaves were sampled according to the categories described before. However, the age of the apical leaves now was 5-10 days, the intermediate leaves 40-50 days, and the oldest leaves ca. 60 days old, as compared with the ages, 1-3, 5-10 and ca. 20 days, respectively, of the leaves sampled in April. The content of fungicides in leaves sampled in May showed the same correlation with leaf age and injection date as the data from the April samples. To examine the movement of the fungicides towards the top of the tree, intermediate leaves were sampled on 20 May from 4-m height.The content of fungicides in leaves from trees injected in autumn or spring was 21 or 5 μg MBC/g fresh wt, and 20 or 23 μg TBZ/g fresh wt, respectively. In comparison with the intermediate leaves from 2 m height (Table 2), the content of MBC was similar while that of TBZ was considerably lower at the top.

Phytotoxic symptoms were produced by both compounds in trees injected in the spring. Two weeks after treatment some of the leaves became curled and necrotic; in a few flowers browning of the petals occurred. Later in the season, in the TBZ-treated trees, veinal discoloration of the midrib and its vicinity was apparent in some leaves in the lower half of the tree; similar discoloration was observed, and even more frequently, on autumn-injected trees (Fig. 1-A). In some shoot apices in trees treated with TBZ at both seasons, leaves were more elongated than the normal ones. No phytotoxic symptoms were observed during spring in trees injected with MBC in autumn.

Pear scab infection, caused by *Venturia pirina* Aderh., during the spring was low due to the unfavorable weather conditions. Sparse late infections on mature leaves were observed in the summer. Both primary and late scab infections were confined to the untreated trees only.

Experiment 2.—The time necessary to complete the injection of MBC solution varied according to the stage of growth at the time of injection. The infiltration at dormancy took 2-4 h, compared with 5-10 min in the trees partially covered with leaves,

TABLE 2. Concentration of fungicides in pear leaves from various positions on new terminal shoots, of trees injected at different times

Fungicide	Date of injection	Fungicide content (μg/g fresh wt) of leaves[a] from injected pear trees					
		Sampled 9 Apr 1973			Sampled 20 May 1973		
		Basal	Intermediate	Apical	Basal	Intermediate	Apical
MBC[b]	22 Nov. 1972	64 (43-86)[c]	21 (11-37)	4 (2-6)	50 (35-74)	20 (4-48)	8 (4-12)
MBC	5 March 1973	9 (4-12)	3 (0-4)	0 (0-1)	10 (5-15)	5 (3-8)	3 (0-4)
TBZ[d]	22 Nov. 1972	46 (7-102)	2 (0-11)	1 (0-6)	123 (91-160)	81 (43-120)	62 (34-98)
TBZ	5 March 1973	5 (0-11)	0	0	39 (11-61)	62 (15-133)	23 (14-34)

[a]Leaves were collected from new terminal (extension) shoots of 20-40 cm in length at a height of ca. 2 m on the dates indicated. Leaves were sampled according to position on the shoots: basal (oldest), apical (youngest) and intermediate.

[b]MBC = methyl 2-benzimidazolecarbamate.

[c]Data are expressed as μg fungicide/g fresh wt leaves. The figures are mean of five replicates (from different trees), each of 5 g leaves. The figures in brackets represent the ranges.

[d]TBZ = thiabendazole.

TABLE 3. Concentration of methyl 2-benzimidazolecarbamate in wood tissue of pear trees injected at different times

Time of injection (1973)	Dose (g/limb)	Sampling date (1973)	Height above injection point (cm)							
			50	100	150	200	250	300	350	400
dormancy (2 February)	1.25	14 Feb.	23[a]	0	0	0	0	0	0	0
		12 March	–	0	0	0	0	0	0	0
		29 March	126	29	0	0	0	0	0	0
		15 April	40	2	0	0	0	0	0	0
		6 July	7	4	0	0	0	0	0	0
	5.00	14 Feb.	239	0	0	0	0	0	0	0
		12 March	–	0	0	0	0	0	0	0
		29 March	192	33	0	0	0	0	0	0
		15 April	137	43	1	0	0	0	0	0
		6 July	59	4	1	0	0	0	0	0
after bud burst (29 March)	1.25	29 March	203	130	26	0	0	0	0	0
		15 April	36	23	18	27	25	17	19	3
		6 July	34	47	35	29	31	0	0	0
	5.00	29 March	99	64	25	0	0	0	0	0
		15 April	78	73	64	54	26	12	9	6
		6 July	140	160	148	115	45	6	0	0

[a]Data are expressed as μg fungicide/g fresh wt of wood and are averages of five replicates, 0.5 g wood tissue each.

injected on a hot, dry, windy day.

Primary distribution of MBC in limbs of trees injected at dormancy was limited to 50 cm above the injection point (Table 3). A limited secondary distribution occurred after bud burst. With spring injection, the primary distribution of MBC was not confined to the vicinity of the injection point, and during the next 2 wk the fungicide reached the top of the tree due to secondary distribution (Table 3). The injected fungicide was also forced downward, and was evident in segments sampled from 50 cm below the injection point.

Several leaf samples collected in the early spring, up to 15 Apr, did not reveal the presence of MBC. Eleven weeks later (6 Jul) leaves were sampled from heights of 3 and 5-6 m. In trees injected at dormancy, only traces of MBC were detected. In the spring-injected trees the content of the fungicide in the leaves differed according to the dosage applied and the height of the sampled leaves. With the low dosage the MBC concn at 3 m and 5-6 m was 11 and 0 μg/g and with the high dose 90 and 9 μg/g fresh wt, respectively.

When the injected limbs were cut into segments, a distinct discoloration at the center, along the limb, was apparent (Fig. 1-B). Hydrochloric acid controls did not show similar symptoms. Bioassay tests revealed

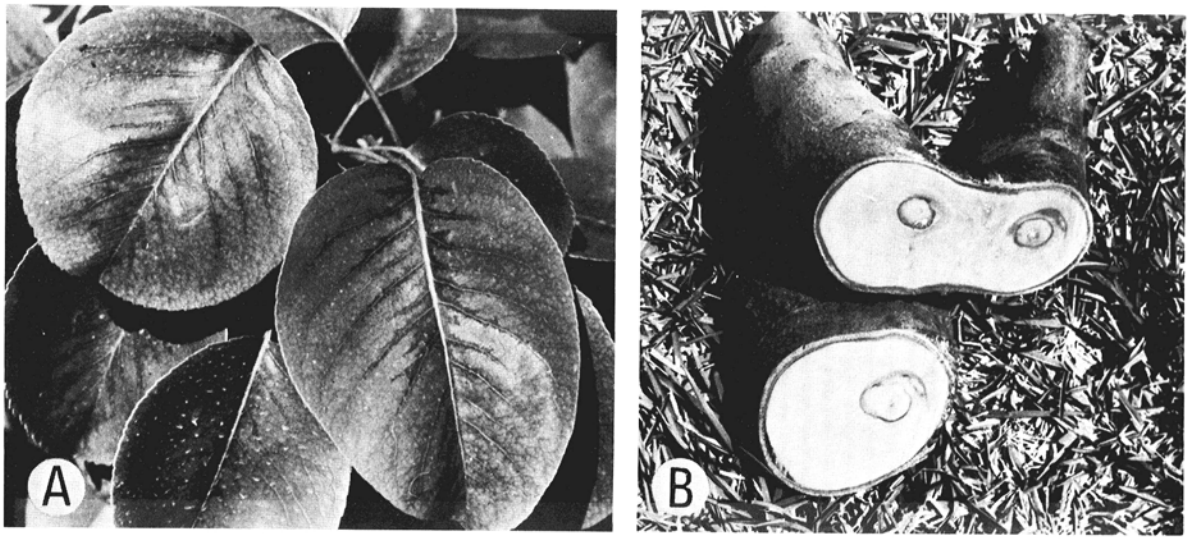

Fig. 1-(A, B). A) Phytotoxic symptoms in leaves of pear trees injected with thiabendazole. **B)** Discoloration in the wood of pear tree injected with MBC. Sections below and above the branching.

that a high amount of MBC was present in the discolored tissue.

Scab was not detected in this plot at any time during the season due to the unusually dry spring.

DISCUSSION.—Injection of fungicides into trees to control foliar diseases may have several advantages over conventional spray treatment. With a spray program leaves emerging between two sprays are not protected during their first stages of growth. The introduction of a substantial amount of fungicide directly into the conductive vessels of the tree might provide the plant with long-lasting protection by continuously supplying the new growth with a fungitoxic compound. With the injection method, waste of an expensive toxicant and environmental pollution are minimal.

Injection of MBC at four growth stages resulted in different rates of translocation and accumulation of the toxicant in branches and leaves. When trees were injected in autumn a rapid secondary distribution process occurred, as was evident from the high amounts of MBC detected in the leaves before they dropped. Next spring, since the toxicant was in the vicinity of the buds, it was readily taken up by the emerging leaves. With both spring injections there was a lag between injection and accumulation of the fungicide in the emerging leaves, due to a slow secondary distribution. Later, when the trees were fully covered with leaves, higher amounts of MBC were translocated into the new emerging leaves, apparently because of greater transpiration at that time. When trees were injected in dormancy the primary distribution was restricted, presumably because of the lack of transpiration during injection.

No subsequent distribution occurred during dormancy, and when trees resumed growth, only negligible migration was recorded. Similarly, Gregory et al. (3) reported that the immediate distribution of azosulfamide dye in red oak trees injected at dormancy, was less rapid and of a smaller extent than in those injected in September. The failure of dormancy treatment emphasizes the dependance of secondary distribution on the primary spread.

Accumulation rate of TBZ in the young leaves was slower than that of MBC inspite of its five-times-greater dose. However, TBZ accumulation during the growing season exceeded that of MBC, apparently due to depletion of MBC reserves in the trunk.

The phytotoxicity symptoms revealed on some leaves during the season reflect the accumulation of fungicide in the leaves. In TBZ-treated trees, more affected leaves were detected and the intensity of symptoms was augmented as the season progressed. However, incidence of the affected leaves in the various treatments was low and apparently without any significance.

Pressure injection of benzimidazole fungicides provided the new foliage with increasing amounts of toxicant which might be sufficient to control foliar diseases. Our results indicate the validity of this method for control of a foliar disease. The efficiency of the injection treatment needs to be further evaluated under high inoculum pressure of pear scab.

LITERATURE CITED

1. FILER, T. H. JR. 1973. Pressure apparatus for injecting chemicals into trees. Plant Dis. Rep. 57:338-341.
2. FILER, T. H. JR. 1973. Suppression of elm phloem necrosis symptoms with tetracycline antibiotics. Plant Dis. Rep. 57:341-343.
3. GREGORY, G. F., T. W. JONES, and P. MC WAIN. 1971. Injection of benomyl into elm, oak and maple. U. S. Dep. Agric., For. Serv., Res. Paper NE-232. 7 p.
4. HELBURG, L. B., M. E. SCHOMAKER, and R. A. MORROW. 1973. A new trunk injection technique for systemic chemicals. Plant Dis. Rep. 57:513-514.
5. HIMELICK, E. B. 1972. High pressure injection of chemicals into trees. Arborist's News 37:97-103.
6. JONES, T. W., and G. F. GREGORY. 1971. An apparatus for pressure injection of solutions into trees. U. S. Dep. Agric., For. Serv., Res. Paper NE-233. 7 p.
7. MC COY, R. E. 1972. Remission of lethal yellowing in coconut palm treated with tetracycline antibiotics. Plant Dis. Rep. 56:1019-1021.
8. PINKAS, Y., E. SHABI, Z. SOLEL, and A. COHEN. 1973. Infiltration and translocation of thiabendazole in apple trees by means of a pressure injection technique. Phytopathology 63:1166-1168.
9. SCHWARZ, R. E., and S. P. VAN VUUREN. 1971. Decrease in fruit greening of sweet orange by trunk injection of tetracyclines. Plant Dis. Rep. 55:747-750.
10. SHABI, E., Y. PINKAS, and Z. SOLEL. 1973. Pressure injection and systemic distribution of benzimidazole fungicides in apple and pear trees. Phytoparasitica 1:130 (Abstr.).