

Infiltration and Translocation of Thiabendazole in Apple Trees by Means of a Pressure Injection Technique

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ABSTRACT

A method for administering fungicides into apple trees using a pressure injection technique is described. Ten or 2.5 g of thiabendazole (TBZ) dissolved in acidified water was infiltrated into the trunk of 16-year-old apple trees. The fungicide accumulated initially in the vicinity of the injection point, forming a reservoir of TBZ inside the plant.

Subsequently, a secondary distribution occurred, resulting in a gradual upward migration of the fungicide. Forty-two days after injection of the higher dose, TBZ was detected in the top branches and leaves of the tree.

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The translocation of systemic fungicides in herbaceous plants as opposed to woody ones is a well-established fact. Several studies have demonstrated translocation of systemic fungicides in potted seedlings of young trees after either soil or stem applications (4, 7, 11). However, disease control in woody trees by soil and trunk applications has, in general, proved to be without significant success (2, 6, 9). Trunk applications of fungicides have been accomplished principally by gravity flow infiltration of fungicide solutions into holes drilled into the trunk, as described by Schreiber (8) and by Schwarz & Van Vuuren (10). Recently, a method for injecting tetracycline under pressure into trunks of sweet orange was described (10), but no data were presented.

The following research was undertaken to explore the use of pressure injection as a means of introducing fungicides into xylem vessels, and to study their translocation within the tree. Apple trees and the systemic fungicide thiabendazole (TBZ) were selected as the components of this model system.

MATERIALS AND METHODS.—Apple trees (*Malus sylvestris* Mill.), cultivar 'Golden Delicious', 16 years old, were used in all experiments. Each tree had been pruned to develop four main branches on the main trunk approximately 50 cm above soil level.

To improve the solubility of TBZ (1), technical grade TBZ was dissolved in 7% hypophosphorous acid to provide a stock solution with a final concentration of 20% (w/v) at pH 1.88. Further dilutions of the stock solution, containing 10,000 and 2,500 $\mu\text{g}/\text{ml}$ of the fungicide at pH 2.3, were injected into the trunk using the following procedure. Five hundred ml of the fungicide solution was forced with a hypodermic syringe into a 40-cm-long piece of surgical latex tubing [I.D. 6.3 mm (0.25 in), wall thickness 2.4 mm (0.094 in)] that was closed with wire at one end. The solution introduced into the tubing formed a bulbous protrusion which exerted a pressure of 1.4 atm. The end of the tubing which received the syringe was closed with a clamp, the syringe was then removed, and a plastic wall plug was inserted. Two holes, 11 mm in diam and 60 mm deep, opposite one another but in different planes, were drilled into the trunk 35 cm above the soil

surface. The tube-end containing the plug was carefully tapped into the bore-hole, after which the clamp was removed (Fig. 1). In this way, a 500-ml charge containing either 2,500 or 10,000 $\mu\text{g}/\text{ml}$ TBZ was injected under slow continuous pressure into each of the bore-holes. Infiltration of the two 500-ml charges was completed within 15-30 min.

At various time intervals, the trees were analyzed to determine the location and concentration of TBZ. In each case the four main branches of three trees were cut with a chain saw into 5-cm segments, 0.5 m apart, starting 25 cm above the injection hole.

The concentration of the fungicide in the wood was determined as follows. Holes were drilled all over the segments, and the sawdust borings were collected and mixed to form a representative sample. The sawdust was dried at 90 C and reduced to a fine powder (40-mesh) in a Wiley mill. Aliquots of 0.5 g were mixed thoroughly with 10 ml melted water agar (2%), and the slurry was poured into 5-cm diam petri dishes. The dishes were left overnight at 2 C to allow uniform diffusion of TBZ into the agar. Six agar disks, each containing about 25 mg of sawdust, were removed with a 13-mm cork borer and bioassayed for TBZ content with *Verticillium dahliae* Kleb. (3). In each experiment, known amounts of TBZ were assayed similarly to prepare a standard curve.

In preliminary tests the sawdust was homogenized in methanol (10 ml/g wood) with an Ultraturax for 2 min and kept overnight. The extract was filtered, evaporated to dryness, resuspended in methanol, and centrifuged at 20,000 g for 15 min. Aliquots of 50 μl of the supernatant liquid were placed on antibiotic assay filter paper disks and bioassayed against *V. dahliae*.

Determination of TBZ in the wood with both methods yielded similar results. Therefore, because of its simplicity, the direct assay of sawdust in agar was used throughout these experiments. However, TBZ content in leaves was determined by the methanol extraction procedure. The fungitoxic material in wood was ascertained to be TBZ by comparison with an authentic TLC sample by thin-layer chromatography (TLC). The TLC plates (Silica Gel GF 254; Merck & Co.) were

developed with the solvent system ethyl acetate-benzene (19:1, v/v), dried, and the spots viewed with ultraviolet light.

RESULTS.—The results (Fig. 2) revealed that the distribution of TBZ in the wood is a function of time and dose. Immediately after injection, TBZ was detected mainly 25 cm above the injection hole in both high- and low-dosage treatments. However, with the higher dose, the fungicide could be detected even at a distance of 125 cm from the site of injection. Three days later, TBZ had not migrated significantly farther. However, 21 days after the high-dosage treatment, TBZ was detected along the entire length of the main branches up to 275 cm from the point of injection. During the subsequent 21-day interval, the fungicide reached the very top of the tree, and its accumulation along the main branches continued intensively. Occasionally, one of the four main branches in the treated trees failed to reveal the presence of fungicide. It is assumed that this was due to its position with respect to the injection point, and thus was not considered in the calculations of fungicide level in the tissue.

Leaves were collected from the tops (ca. 3.5 m from the site of treatment) of 12 main branches, and assayed for TBZ content. The fungicide was not detected in any of the leaves sampled from trees treated with the low dose of TBZ. Following the high-dosage treatment, TBZ was not recovered until the 21-day sampling, and even then it was traced in leaves from two branches only. However, 42 days after treatment, TBZ was found in leaves taken from eight of the 12 branches tested, the amount of fungicide ranging from 18 to 132 $\mu\text{g/g}$ fresh wt.

When the volume of the treatment solution was increased to 10 liters per tree, while maintaining the same fungicide dosage, no change in the distribution of TBZ in the wood was noticed. Samples sliced immediately after treatment 25 cm below the injection hole revealed that the fungicide had also infiltrated downward. Phytotoxic symptoms were not evident at any time during the experiments, nor did they appear in a tree injected with a large dose of 40 g (40,000 $\mu\text{g/ml}$) of TBZ and checked 2 months after treatment.

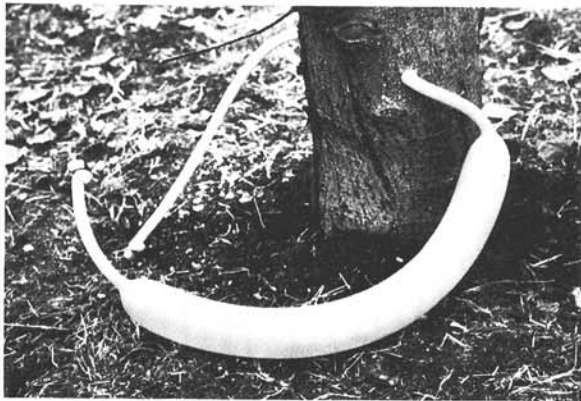


Fig. 1. Pressure injection of thiabendazole (TBZ) into the trunk of an apple tree, from latex tubing containing the fungicide solution under pressure.

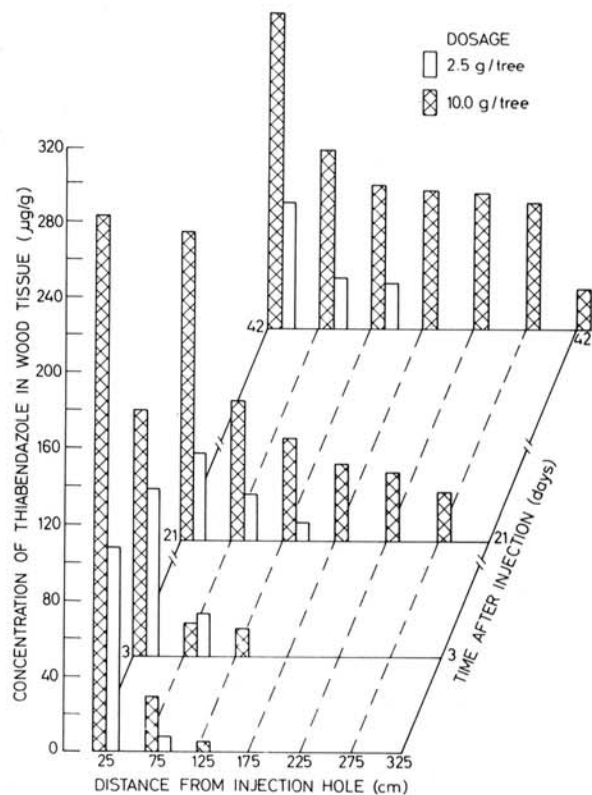


Fig. 2. Distribution of thiabendazole (TBZ) in apple trees after pressure injection into the trunk. Each figure is an average of 9-12 replicates (3-4 branches \times 3 trees).

In another series of experiments, TBZ solutions were pumped through excised 3-year-old apple tree branches, and the infiltrated solutions were collected at the opposite end. Solutions of 400 $\mu\text{g/ml}$, at a pH of 3.4, were prepared by diluting the acidic stock solutions with either water or a citric acid- Na_2HPO_4 buffer. With the aqueous solution, the concentration of the fungicide in the recovered solution dropped from 400 $\mu\text{g/ml}$ to 13 $\mu\text{g/ml}$, and the pH of the solution rose to 6.7. This pH value was similar to the normal pH of the wood sap of untreated apple branches. When the fungicide was dissolved in the buffer solution having a pH of 3.4, the concentration and the pH of the collected solution were 110 $\mu\text{g/ml}$ and 3.5, respectively.

DISCUSSION.—Successful disease control depends upon the concentration of the toxicant that ultimately reaches the site of infection. With trunk injections, a large dose of the fungicide must be administered through the small area of the injection point to attain a fungitoxic level in all parts of the tree. The solubility of TBZ in water is low, 50 $\mu\text{g/ml}$; thus, to infiltrate our test levels of the fungicide (10 g and 2.5 g) would have required 200 or 50 liters of water per tree. However, by putting TBZ into solution at pH 2.3 it was possible to get these relatively high doses into trees in 1 liter of solution.

Since the solubility of most fungicides in water is very low, their application by the trunk injection method is not practical. Fungicides of low solubility must be solubilized

in a nonphytotoxic medium. In our experiments the acidic formulation did not result in any phytotoxic symptoms. The water-soluble tetracycline or phosphamidon have been successfully administered by gravity flow. The former decreased fruit greening of sweet orange which is probably caused by a mycoplasma-like organism (10), and the latter controlled the pine processionary caterpillar (*Thaumetopoea wilkinsoni*) on pine trees (5).

The injected TBZ accumulated mainly in the vicinity of the application point. We believe that the fungicide precipitates there due to the rise in pH, as was demonstrated in detached apple branches. We contend that in the course of time, a process of secondary redistribution occurs which potentiates migration of the fungicide toward the top of the apple tree. Since the movement of TBZ is apoplastic, the fungicide is carried by the transpiration stream to the apex of the branches into twigs and leaves. The large volume of water ascending in the conducting vessels (ca. 100 liters/day per tree) dissolved and carried the fungicide upwards despite its low solubility.

The pressure injection method enabled the infiltration of a large dose of TBZ into the trunk, thus forming an internal reservoir of fungicide available for subsequent redistribution.

Our results have demonstrated long-distance translocation and distribution at fungitoxic levels of a systemic fungicide in a tree. It appears that a single treatment of this kind might provide long-lasting protection against pathogenic organisms.

Control experiments against several diseases in fruit trees are currently being conducted using a modified injection technique. The fungicide solution is contained in a metal tank and is being injected into the trees by the force of compressed CO₂.

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