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FLUID DELIVERY IN INJECTED RING-POROUS TREES[1]

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Abstract. In ring-porous trees such as elm, oak, and ash, trunk injections of fungicides for control of vascular wilts should be specifically directed to the single outermost growth ring of wood. It transports the most water, and is the first to become infected by fungal wilts. Shallow-pit injection taps into this target tissue, and is enjoying widespread use among arborists and researchers. Evidence to its effectiveness comes from theoretical, laboratory, and clinical studies. The need now is to quantify spread of trunk-injected fungicide in the crown. Gas chromatography and mass spectroscopy clearly detect thiabendazole (TBZ) from samples containing 1 part per million TBZ. This sensitive means of detecting fungicide in crown tissue is essential to optimize TBZ dosages, minimize injection injury, and to detect TBZ persistence in outermost wood of twigs years after injecting the trunk.

Résumé. Chez les arbres à zone poreuse tels les ormes, les chênes et les frênes, l'injection de fongicides dans le tronc pour contrôler les flétrissures vasculaires devrait être réalisée spécifiquement dans le dernier cerne annuel. Celui-ci transporte le plus l'eau, et il est le premier à être infecté par les flétrissures fongiques. Des trous d'injection peu profonds atteignent directement cette localisation et sont utilisés grandement par les arboriculteurs et chercheurs. L'évidence de leur efficacité vient d'études théoriques, en laboratoire et sur le terrain. Le besoin maintenant est de quantifier la vitesse de dispersion dans la cime du fongicide injecté dans le tronc. La chromatographie en phase gazeuse et la spectroscopie de masse détectent clairement la thiabendazole (TBZ) dans des échantillons contenant une partie par million de TBZ. Ce moyen très précis permettant de détecter le fongicide dans la cime est essentiel afin d'optimiser les dosages de TBZ, de minimiser les blessures dues à l'injection et de détecter la persistance du TBZ dans les cernes annuels des années antérieures à l'injection.

Background for some of the ideas in this paper has been introduced by Drs. William Chaney, Jay Stipes, and Kevin Smith earlier in the Symposium. Convergence of their ideas with points brought up in this article shows that optimizing systemic chemical treatments for trees requires input from several disciplines, including that of the practicing arborist. While Chaney and Stipes concentrated on functional properties of wood and gaps in our knowledge of tree behavior, Smith laid the foundation for promoting trunk injection to deliver chemicals into trees.

This paper has four aims: 1) to summarize hydraulic principles critical to predicting wood function; 2) to examine current evidence for the distribution of fluid flow in ring-porous trees; 3) to show how this research is applied to developing systemic protocols; and 4) to outline the direction of future research in this area.

Hydraulic Principles

Subject to the usual amount of dispute over hypotheses, it is widely held that water travels up trees by being pulled through the xylem (14). Suction is not involved, since no compartment involved in water ascent contains gas at significantly less than atmospheric pressure. Instead, conducting vessels are filled with water, comprising a column in the lumen of vessel elements. Water molecules evaporate at the leaf surface. Hydrogen bonds promote cohesion between water molecules ensuring that as leaf water evaporates, adjoining water molecules are pulled up after it. In this way there is an upward pulling force imposed on the water column by the high affinity of the atmosphere for water presented on the leaf surface. At the same time, gravity imposes a downward force on the water column. The effect of these two opposing forces is to stretch the water column between leaves and ground level, just as a band of rubber molecules is

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stretched when simultaneously pulled from above and below. Rather than being sucked up the tree by a partial vacuum, the water column is under tension as its molecules are individually pulled up into the atmosphere during transpiration at the leaf surface.

Against this transpirational backdrop for motive force, investigators have discovered new relationships between xylem anatomy and tree function, some of which Dr. Chaney pointed out earlier. By combining anatomical measurements with the principles of fluid dynamics, a hydraulic component of tree development has been found (12).

The contribution of fluid dynamics to xylem function and the ultimate ability of a tree to take up injected fluid begins by comparing water transport in tree vessels with that in glass capillary tubes (Fig. 1). If we could label a row of water molecules across a glass capillary (Fig. 1a), and the water is then propelled upward, the advancing front will describe a parabola (Figs. 1b & c). Hydrogen bonds between water and glass force water adjacent to capillary walls to adhere to the glass, rather than moving up the tube. Hydrogen bonds also promote clinging between water molecules. Water molecules clinging to the stationary ones adhering to the capillary wall move more slowly than do those farthest from the wall. Thus, water in the center of a capillary will climb faster than will the water nearer the edges (Fig. 1c).

Wider capillaries, or pipes, will allow a far greater flow rate that will narrow ones. Measurements have shown flow rate to be proportional to the radius of the water column taken to the fourth power, as expressed by the Poiseuille equation (13):

Equation 1.

$$R \propto r^4$$

where R = flow rate through capillary
r = inside radius of capillary

Water flow through vessels in wood conforms closely to Equation 1. Despite complicating factors such as pit size and frequency in vessel walls, and perforation plates between vessels (5), most water conduction up a tree can be accounted for by the Poiseuille equation (Eq. 1).

To predict the level of flow through wood, we need to examine the diameter of conducting elements (vessels and tracheids). Wider elements will accommodate far greater fluid flow than will narrow ones. The difference is striking; an 80 μm-wide element is only four times wider than one of 20 μm, yet will permit 256 times (4^4) the flow rate found in the narrower element. In many trees, especially ring-porous species, conducting vessels can range from 25 μm to 300 um within a single individual. Thus some elements will transport thousands of times more water than will others in the same tree.

Distribution of Water Flow in Ring-porous Trees

Knowing the location of functioning (conducting) elements is critical to the problem of tree injection. The goal is to use the most active conducting area to distribute chemicals away from injection ports. Most injection protocols fail to deliver fluid to the most actively conducting part of the tree. Results are needlessly long injection times, and uneven chemical coverage in the crown. Over the past three years, we have focused on the most useful target tissue for tree injections, by applying hydraulic principles and testing their value in predicting fluid movement in tree trunks.

Two major wood types among dicotyledonous trees are recognized by plant anatomists. Diffuse-porous trees such as maple, willow, and linden, produce wood with vessels of similar diameter scattered throughout each annual growth ring

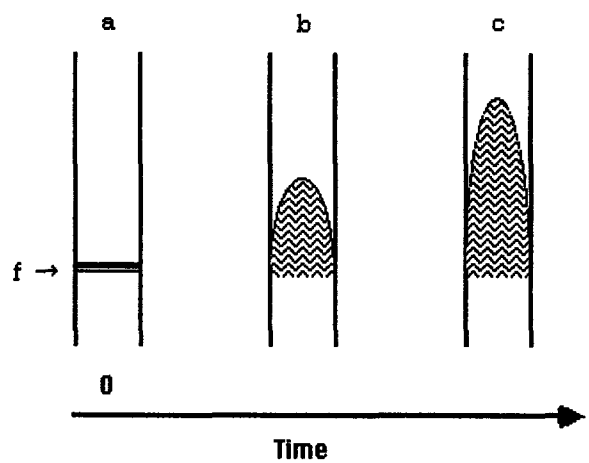


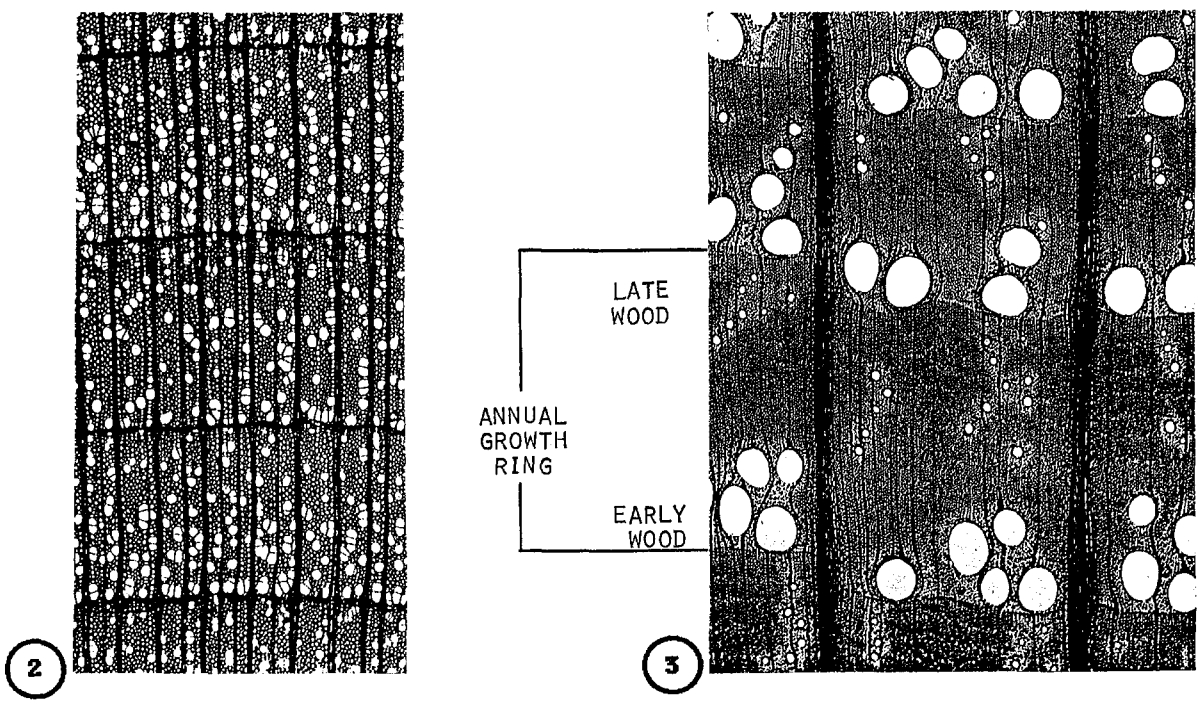
Figure 1. Pattern of water ascent through a glass capillary tube over time. f = initial front of water molecules at time zero.

(Fig. 2). On the other hand the widest vessles are concentrated in the earlywood of ring-porous species (Fig. 3). Chestnut, elm, oak, and ash fall within this group. It is no coincidence that ring-porous trees are tragically vulnerable to vascular wilt diseases.

Certainly the wide vessels of ring-porous trees can theoretically (Eq. 1) handle hundreds of times more fluid flow than can narrow vessels, to which diffuse-porous trees are restricted. However, this advantage comes at the expense of safety (13). High water tensions in late summer and fall, conspire with winter freezing (5) to form bubbles in vessels. According to one idea, enough wide vessels are blocked by those two mechanisms that a given annual growth ring of ring-porous wood functions only for one growing season, then is permanently blocked. Zimmermann (13, 14) has designated this strategy as "throw-away" xylem activity in which conductive rings are discarded annually, and fluid flow must depend on the single growth increment which has not yet experienced a winter. Narrow vessels are much less prone to blockage, and there are more of them to survive adverse abiotic effects.

To counter the notion that ring-porous trees are restricted to one functioning growth ring, other workers point to dye experiments which show colored fluid moving through rings up to eight years old (1, 2). This forces us to re-evaluate the "throw-away" hypothesis in an effort to reconcile it with data from dye distribution experiments.

The most frequently cited evidence that water transport in ring-porous trees occurs primarily in the outermost growth ring is work done by Huber (6). By measuring migration of heat pulses in trunks, he showed that peak velocities in ring-porous trees are often ten times greater than in diffuse-porous species. He then proposed a link between velocity of fluid flow and wood structure:



Figures 2 and 3. Cross section of diffuse-porous (2) and of ring-porous (3) wood as seen under the light microscope. Conducting elements are much wider in ring-porous trees, but there are far fewer of them. When one fails due to injury, water shortage, or freezing, the effects are far more devastating in ring-porous trees than in diffuse-porous ones. (Modified from Raven et. al. 1981. Biology of Plants)

velocity of flow is proportional to the volume of fluid moving up the trunk, divided by the amount of wood involved in conduction (Eq. 2)

Equation 2

$$V \, a^{k_{vol}} / A$$

where v = velocity of water conduction
k_{vol} = volume of fluid transported
A = cross sectional area devoted to conduction

The greater flow rate (v) in ring-porous trees results either from a greater volume (k_{vol}) transported in these species, or a smaller area (A) devoted to moving water up ring-porous trunks. To discount the first possibility, Huber supposed that volume moving through ring-porous and through diffuse-porous trees was similar since both grew side by side, supported by the same soil and climate. Thus, we designate the volume of fluid moved as a constant (Eq. 2) when comparing the two wood types.

Advances in plant physiology over the past fifty years show that when comparing individual trees, there are several variables contributing to volume of transported fluid in the trunk. Differences in total leaf surface area, stomatal resistance, and cuticular transpiration all lead to the conclusion that a constant volume of transported fluid is no longer acceptable on physiological grounds.

The area engaged in active water conduction up trunks of ring-porous trees is now known to be quite small. Current evidence (3) points to the single outermost growth ring as the only one whose wide vessels (Fig. 3) function in water conduction. Those of 2-year old, or older, wood are permanently blocked by embolisms and later tyloses, as suggested by Zimmermann (13). Nevertheless, dye experiments confirm that some fluid is conducted by older growth rings (2, 3). However, only narrow, latewood (Fig. 3) vessels carry fluid through older rings, and their small diameter prevents them from transporting significant amounts of water. In ring-porous American elm, the outer ring accommodates over 90% of the water moving through the trunk (3). The remaining 10% makes its way up the narrow latewood elements in older rings, which are less prone to blockage and thus function for several years. Dye uptake experiments do not distinguish between wide vessels allowing large flow rates, and narrow ones.

We thus reconcile the theoretical prediction that wide, hydraulically significant vessels only conduct water for one season, with the often reported result that tracer dyes are found in rings older than one year of age. Three lines of evidence indicate the outer growth ring's overwhelming importance to fluid ascent in ring-porous xylem. First, removing the outer ring decreases hydraulic conductance of the entire trunk to less than 10% of that found in unaltered control trunks (2). Second, tracer dyes show that material flows through several growth rings but that only the outermost one provides conductive earlywood (Fig. 3). Finally, some water is transported in older rings but it is hydraulic insignificant compared to the transport rate accommodated by conducting earlywood vessels (3). The biological significance of minimal water flow in older rings may be as a safety reserve, or to help re-establish the water column in spring (Pieter Baas, pers. comm.).

Developing an Injection Protocol

Injecting trees with fungicides is a procedure which promises to be with us for some time. The severity of vascular wilt diseases, and the frequency with which new aggressive strains of pathogen arise in nature (10) mandate the need to combat pests like Dutch elm disease from inside the tree. Until the development of trees which resist disease as adults, as well as the more accessible (and more frequently advertised) sapling phase, drugs injected into trees will remain a major line of defense against pathogens attacking large specimens most valuable in landscape architecture (8). Research summarized in the first two sections of this paper allowed us to develop an injection protocol for ring-porous trees based on firm scientific principles (9). We have been most successful in applying our findings toward controlling Dutch elm disease (4), the program upon which we base the discussion below.

Having reviewed the case for the overwhelming significance of the outermost growth ring to water transport, and its vulnerability to pathogens entering from the bark, a technique was developed to deliver fungicide to the outermost ring (9). The

method is designated as "shallow-pit" injection because it deviates from the usual practice of drilling at least 40 mm (ca. 1.5 in) into the trunk. Instead, holes are drilled into target tissue 3-5 mm beneath the vascular cambium (Fig. 5). This is the most conductive wood, insuring greater spread of fungicide throughout the crown. Re-usable injectors are nestled in holes whose edges swell as they get wet with fungicide (Arbotect 20-S). Thus swollen, the edges firmly hold injectors in place while several gallons of pressurized (30 psi) fungicide are forced into the trunk. Mature elms, between 25 and 90 years old, take up the required dosage within 30 minutes. In contrast, several hours are usually needed for fluid to percolate into an elm injected through deeper ports.

The problem with most insertable "T"—shaped injectors is that they are either placed at least 4 cm into the trunk, or are put into holes drilled at least that deeply (Fig. 4). Either way, fungicide ends up bypassing the outermost ring and being forced into non-functioning older wood. Growth rings are often less than 3 mm (ca. 1/8 in) wide; precise drilling is required to avoid going too deeply. The price of injecting even millimeters too deep is greatly extended time needed to take up the fungicide, sporadic crown coverage, and inconsistent control of vascular wilts.

On the other hand shallow-pit injection delivers fluid more superficially (Fig. 5), channeling fungicide exclusively to the most recent growth increment. That narrow band of wood is most conductive and most prone to infection. It is here that fungicide is needed the most. Shallow-pit injection has supported a 100% survival rate among our pilot population of over 50 large American elms in Massachusetts. Over the same eight years, uninjected control populations in the same vicinity have been decimated from a yearly mortality rate of over 20%. For elm populations treated with conventional injectors (Fig. 4), annual loss of 5-10% is common.

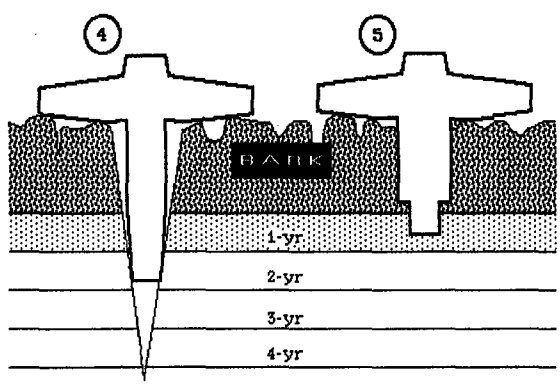
The shallow-pit protocol is an important advance over earlier methods of injecting tree trunks. It is now practiced by professional arborists in Maine, New Hampshire, New York, Virginia, North Carolina, and in Massachusetts where it originated. Private estates in the Midwest are using it, and the US Forest Service is testing its utility on oak and even conifers (by no means ring-porous!) in the South. So much fungicide reaches living tissue by this method, that reductions in recommended dosages for commercial preparations such as Arbotect 20-S must be considered. Our present research effort is aimed at determining the most effective dose of trunk-delivered fungicide for control of Dutch elm disease.

Future Research

We have shown how hydraulic theory and laboratory work point to the usefulness of shallow-pit injection. The technique's success in clinical studies with mature elms (4, 9) testifies to its effectiveness in controlling Dutch elm disease. Confirmation should strengthen as use of shallow-pit spreads among arborists and researchers.

The pivotal point in any injection is coverage. How well does injected material spread away from injection points, to reach distant sites in the crown and root systems. Crown coverage is critical for control of diseases carried by bark-boring insects. For example, twig crotches are feeding sites for the European elm bark beetle *(Scolytus multistrialus)*. Fungal spores easily rub off beetles' bodies, enter feeding wounds and infect the tree with Dutch elm disease (7). To control the condition, fungicide must reach the primary infection sites in the crown.

Recently, Stennes and French (11) squarely addressed the issue of crown coverage achieved



Figures 4 and 5. Schematic views of "T"-shaped injectors seated in a trunk cross section during injection. 4. Most commonly used injectors block the outermost (1-yr) ring and deliver fluid to 3- and 4-year old wood (at the tip of the injection hole). 5. Shallow-pit injectors target highly conductive 1-year old wood.

by tree injection. They used bioassay to evaluate various injection protocols on American elm. Trees were injected, then twig segments collected from the crown were subjected to attack by cultured pathogen in the laboratory. Those segments that resisted laboratory infection were judged as containing fungicide which had initially been injected at the base of the trunk. Others which failed to inhibit infection must have come from unprotected parts of the crown, not reached by the injections. The rigorous work of Stennes and French (11) contributes greatly to our ability to critically compare injection protocols and the effectiveness of various commercial preparations in controlling Dutch elm disease.

In our laboratory, we are developing ways to chemically analyze crown parts without recourse to bioassay. Living test material is prone to variability all too common among biological systems, especially those spared from intensive agricultural breeding programs designed to impose uniformity. No tree species has been genetically manipulated as much as corn, tomato, soybean, and other crops. A successful analytic method should be sensitive, provide unambiguous results, be rigidly standardized and highly reproducible. We have applied these criteria toward analyzing elm wood for traces of thiabendazole injected into the trunk, using gas chromatography and mass spectroscopy (GC/MS).

Arbotect 20-S is injected into mature elms. Arbotect's water soluble fungicide, thiabendazole hypophosphite (Fig. 6) probably dissociates during metabolism, producing thiabendazole (TBZ) which persists in the tree. Material extracted from elm twigs is run against known standards of TBZ, to quantify crown coverage. First, known concentrations of TBZ are run on the GC/MS to determine how, and when, it appears when plotted by the instrument (Fig. 7). Second, wood from uninjected elms must be spiked with known amounts of TBZ to determine the efficiency of our extraction process. Finally, elm twigs from injected trees contain unknown amounts of TBZ, and extracts from them must be compared to "control" extracts taken from elms never exposed to fungicide. This outline, strengthened by additional controls, will allow us to quantify crown coverage of trunk-injected materials.

Elm twigs (ca. ½-inch diam) are collected with an extendible pruner from a bucket truck. In the laboratory, we strip off the bark, then shave off the outermost wood with razor blades or a steel knife. This ensures that we sample only the outermost wood, unlike bioassays which analyze entire cross sections (Stennes and French, 1987). It is critical that fungicide reach the outermost twig wood since this is the primary infection site (7). Wood shavings are then dried at 80°C and

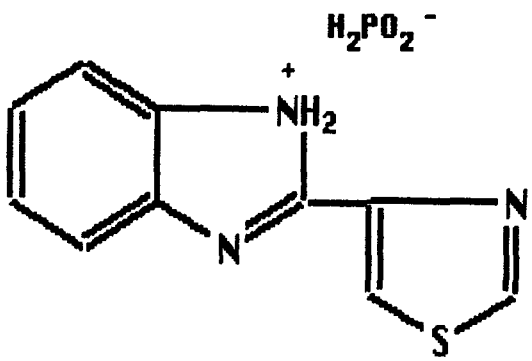


Figure 6. Thiabendazole hypophosphite (2-(4-thiazolyl)-benzimidazole hypophosphite), the fungitoxic component of Arbotect 20-S. Hypophosphite is ionically bonded as a counter ion to TBZ, greatly increasing solubility. In the tree, TBZ-P probably dissociates into insoluble TBZ which persists.

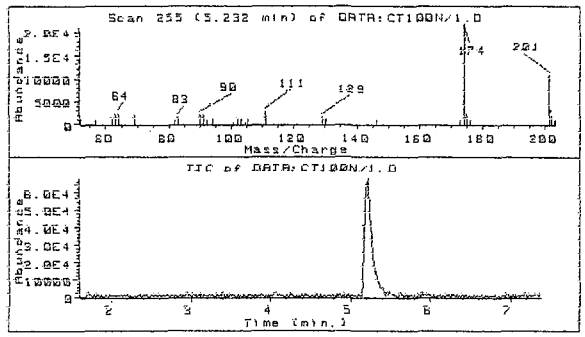


Figure 7. Tandem plots of TBZ provided by the Hewlett-Packard 5988 gas chromatograph/mass spectrometer. Lower plot indicates retention time of just over 5 min in the gas chromatograph. Upper plot shows the relative abundance of TBZ ionization products generated by the mass spectrometer (from the TBZ parent molecule represented by peak in lower plot). Sample was of 1 ppm TBZ in EtAC.

ground into dust using a Wiley mill.

Extracts are made by mixing 5 gr wood dust with 100 ml ethyl acetate (EtAC) and refluxing in a Soxhlet extractor for 4 h. The now-colored EtAC is filtered then vacuum evaporated to dryness (ca. 15 min) before being filtered, capped, and sealed in glass vials where samples are stored until injected into the GC/MS.

The Hewlett-Packard 5988 GC/MS is a tandem instrument. Liquid samples (1 ul) are injected into it, and propelled by helium. Samples are first separated by gas chromatography. Each component in the sample mixture exits the GC column at a specific time. In Fig. 7, TBZ left the column in just over 5 min. That this was a pure sample is evidenced by the lack of other materials retained at different times (Fig. 7, lower plot). Components thus separated from the original mixture are sent into the mass spectrometer where they are bombarded by high energy electrons. Such abuse breaks the parent compound (TBZ in our case) into ionic fragments whose abundance is plotted against molecular weight (comparable to mass: charge ratio when dealing with single-charged species) as shown in the upper plot of Fig. 7. Compounds initially separated in the GC are identified by the cluster of ionization products produced in the MS. Three molecules always appear when TBZ is ionized. Their molecular weights are 201 (unfragmented TBZ), 174, and 129 (Fig. 7, upper plot). These products always occur where TBZ is found, and are absent in EtAC, unhydrolyzed Arbotect 20-S, and untreated elm wood. They constitute the molecular "fingerprint" of TBZ, identifiable through mass spectroscopy. In short, the GC separates compounds out of a mixture, and the MS identifies those compounds.

The GC/MS is a powerful tool for analyzing large numbers of samples such as those needed to document coverage throughout a large crown. It avoids variability inherent in tempermental bioassays, and has already supplied unambiguous results when applied to extracts from elm twigs. Use of this instrument figures strongly in future contributions of this laboratory to three facets of systemic chemical treatments in trees: 1) determination of effective chemical dosages for disease control (shallow-pit injection requires less TBZ than do conventional injections because the fungicide concentrates in the target tissue); 2) decreasing the number of injection ports, to provide adequate disease protection while minimizing trunk damage; and 3) detection of TBZ persistence and transfer into wood produced years after injection. This would open the way for longer intervals between treatments.

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