WOUND RESPONSE OF ULMUS AMERICANA I: RESULTS OF CHEMICAL INJECTION IN ATTEMPTS TO CONTROL DUTCH ELM DISEASE

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Abstract. Concern for injury to elms from chemical injection led to an evaluation of the impact of wounding and exposure to liquid fungicide on the wound response of Ulmus americana. Small elms were injected with thiabendazole (TBZ) plus solvent, its solvent alone, or water. The external symptoms of injury following injection were observed over a 23 month period and the internal response to injury was recorded over a 33 month period from dissection of stems during the growing and dormant seasons. Wounds with water injection had small columns of discolored wood while wounds with chemical injections had large columns of discolored wood. TBZ treatment was toxic to woody tissues and caused the largest amount of discolored tissue in trees injected in May and June. The extensive amount of discolored wood associated with injected fungicide indicated that precautions must be taken such that injections will not limit future distribution of fungicide, decrease storage and transport capacities and predispose trees to other infectious agents.

Chemical injection in attempts to control Dutch elm disease (DED) using a solubilized benomyl derivative was first reported in 1971 (9). The benomyl (methyl 1-[butyl-carbamoyl] benzimadazoly-l-yl carbamate) was "solubilized" using hydrochloric acid. Although injection techniques and formulations have been modified since the early 1970s, all benzimidazole solutions are extremely acidic, and all types of stem or root injection require wounds to get the material into the tree. Stennes and French (23) state that a triple dose of Arbotect-20-S (2-[4-thiazolyl] benzimidazole hypophosphite) (TBZ) may provide protection for at least two growing seasons, reducing the number of required protective injections. However, repeated injections may be required if infected tissues are not removed by pruning, since the materials appear to be fungistatic at points remote from the injection site instead of fundicidal throughout the elm (5, 12).

Discolored and decayed wood associated with wounding has been reported for a variety of hosts

(6, 7, 10, 13, 17, 19, 21, 24, 26, and others), but less information is available on the reaction of woody tissues to chemicals introduced into the tree through wounds. Houston (11) found that a variety of fungicides and other chemicals reduced the amount of discolored and decayed wood in red maple and yellow birch, but Walters and Shigo (27) reported an increase in the amount of injury when tapholes in sugar maples were treated with paraformaldehyde. Elms have been treated with acids, alcohols, and other chemicals in research to understand discoloration associated with DED (4, 8). Broekhuizen (4) found that acids caused more discolored wood than other treatments and the lower the pH, the longer the column of discolored wood. Since 1973, phytotoxicity to woody tissue of elms from acidified benzimidazoles has been reported by several researchers (1, 2, 3, 5, 15, 18, 22). This study was conducted as part of a larger investigation to explore in depth how American elms respond and the extent of injury to them over a 3-year period after wounding and injection with benzimidazole. Since the study was initiated. Andrews, et al. (3) have reported on similar work. Their work provides data similar to ours, but was limited to a 9-month period following injection. Our studies were designed to determine if variation in the extent of injury was associated with the time of injection.

Materials and Methods

Sixty healthy American elms (*Ulmus americana*) from 10 to 15 cm diameter at 1.4 m above the ground (dbh) were chosen for two experiments. Forty-five were injected in July of 1977 and were studied over nearly a 3-year period. These trees were divided into three groups of 15 each; each

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group received a different treatment. The first treatment was Arbotect 20-S (TBZ) injected at the therapeutic rate of 118.3 ml of fungicide concentrate to 4731 ml of water for each 12.7 cm dbh (pH of 2.5). The second treatment was the solvent for Arbotect injected at a rate of 94.6 ml of solvent to 4731 ml of water for each 12.7 cm dbh (pH of 2.0). The solvent rate reflects the fact that it constituted 80% of Arbotect 20-S. The third treatment was sterile distilled water.

The remaining 15 trees were used in a second experiment. They were injected over the growing season on the 15th of each month from May to September 1978 and harvested one year later. Three trees were injected each month; all trees were injected with TBZ at the therapeutic rate.

Injection holes 1 cm wide were drilled with brace and bit 1.3 cm into the wood. There were three wounds per tree at one meter above the ground so that injury below as well as above the wound could be observed. Infusion of liquids into a tree was by gravity using an 8 L reservoir bottle and a hose system with plastic fittings at the injection holes. Each set-up was checked at least once a day until all liquid was gone from the bottles. Injection holes were left open after the injection equipment was removed.

The wounds made for the 3-year study were examined at 1, 11, and 23 months after injection for external differences between the treatments. Since detection of internal differences required dissection of all trees, the 45 trees were harvested over 33 months following injection with one harvest of nine trees (three of each treatment) at 4, 12, 16, and 24 months, and one tree of each treatment at 33 months. Three trees each month from May to September 1979 were harvested in the same manner for the growing season experience. Each tree was cut as close to the ground as possible; the stem was cut in 5 cm discs above and below the wound for the length of the column of discolored wood or to the basal end of the stem. All sections were debarked and brought to the laboratory for further analysis.

The length of the column of discolored wood associated with each wound was measured. The pattern of discolored wood for each wound was traced for each cross section and the surface area was measured using a planometer program on a computer. Since each section was the same height, the volume of discolored wood associated with each wound in each section was determined using the formula for a cylinder. The volumes were added to give total volume of discolored wood associated with each wound. Analysis of variance between treatments, time of removal, etc., was conducted using F tests.

Results and Discussion

Injury associated with injection of chemicals was detected as early as one month after injection. Over the entire 3-year period, exudation of sap (bleeding) (Fig. 1) occurred only from wounds where TBZ or its solvent had been injected (Table 1). The largest number of bleeding wounds were observed at 11 months after injection with both chemicals. By 11 or 23 months after injection many cracks had developed around wounds treated with the chemicals. Cracks were sometimes observed even when callus tissue was present. At 11 months all water wounds had callus tissue present, but only two chemically treated wounds had callus tissue. At 23 months all water-treated wounds had closed while seven TBZ and four solvent treated wounds had not closed.

Both TBZ and solvent treatments were extremely acidic and capable of quickly killing elm tissue before it discolored (2). These chemicals caused



Figure 1. Sap exuding ("bleeding") from elm wound one year following injection with TBZ.

Table 1. Externa	l condition	of inje	cted w	ounds.
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						Num	ber of (Observa	tions ¹				
			W	ater			Thiabe	ndazole))		So	lvent	
MSI	#W	BL	CR	со	WC	BL	CR	со	WC	BL	CR	со	WC
1	45	10	0	0	0	2	0	0	0	37	0	0	0
11	36	0	0	29	7	30	2	1	0	22	3	1	1
23	18	0	0	0	18	1	6	2	11	2	8	1	14

MSI = months since injection, #W = number of wounds observed per treatment, BL = bleeding, CR = crack associated with wound, CO = callus tissue present but wound open, WC = wound closed

^a Some wounds expressed more than one symptom, so that the number of conditions observed may exceed the total number of wounds.

Table 2. Average^a length (cm) and volume (cm³) of discolored wood associated with water, thiabendazole, or the solvent for thiabendazole injected into elms over a 33 month period.

Months since injection	Water		Solu	vent	Thiabendazole		
	length	volume	length	volume	length	volume	
4	12	23	190	515	119	349	
12	11	12	229	652	426	3010	
16	11	12	228	689	463	2970	
24	18	22	282	1350	405	2929	
33	23	51	244	1669	469	4871	

^a The values represent an average of nine measurements except those for the 33 month period which represent an average of three measurements.

Table 3. Mean volume of columns of discolored wood associated with TBZ 12 months after injection.

Month of injection	Mean volume of discolored wood (cm ³) ^a
May	2803 + 857
June	2575 + 529
July	1265 + 269
August	1197 + 372
September	1622 + 715

^a Mean of 9 observations (triplicate wounds to 3 trees) + confidence limits (p 0.05).

significantly greater (p 0.01) internal injury than the water controls at all harvests (Table 2). Differences between the injury caused by TBZ or the solvent alone were not significant at 4 months, but there was significantly more injury (p 0.01) due to TBZ than to the solvent at the twelfth month and

later harvests. Andrews et al (3) have shown that Arbotect 20-S caused more injury than Lignasan BLP (methyl 2-benzimidazole carbamate phosphate), but they did not report the effect of the inactive ingredient of either fungicide. Seventy-one percent of the TBZ-associated columns of discolored wood and 38 percent of the solvent-associated columns of discolored wood extended down to 80 cm below the basal cut, but water-associated columns were never more than 20 cm below the wound. For all treatments, the length and volume of discolored wood essentially stabilized at 12 months unless a bark crack developed associated with a TBZ or solvent injection site. A similar pattern of discolored wood associated with TBZ injection was observed in the trees injected each month from May to September. However, the average volume of discolored wood was significantly larger (p 0.05) for the May and June injections than for the other months (Table 3).

Large variations in the amount of discolored wood occurred between trees of the same treatment at the same harvest time and in some cases, between wounds on the same tree. In the first case, genetic differences in the ability to compartmentalize a wound (20) may account for the results, but in the same tree the differences may be due to differential uptake of the material by different sides of the tree. Larger amounts of discolored wood with May and June injections may be due to differences in the pattern of fluid movements in the tree. In the spring there is a faster rate of fluid movement than later in the growing season, because of an abundance of soil moisture and rapid growth; and the acidic solutions may move further before the pH approaches that of normal sapwood.

Regardless of the time when the injection was done, the injection of TBZ and solvent altered the wound response of these elms compared with the response to water injection. The bleeding and cracking associated only with TBZ- and solventtreated wounds were external symptoms of the extensive internal injury. Tyloses and gums usually limit the length of columns of discolored wood, Scanning electron micrographs by Andrews et al (3) have shown numerous tyloses associated with water-injected wounds, but tyloses were infrequently associated with fungicide-treated wounds. Discolored tissue associated with TBZ and solvent treatments spread extensively in a lateral direction. The fan-shaped discolored areas as viewed in cross section indicate the death of ray parenchyma following treatment with either chemical (Fig. 2). In contrast, the wounded area of water-treated trees were nearly parallel, indicating living ray parenchyma prevented invasion of tissues by microflora (Fig. 3). In almost every case the discolored wood associated with TBZ and solvent injections merged with previously



Figure 2. Section from an elm one year after treatment with TBZ. The treatment-initiated discolored wood has merged with discolored wood present before injection.

discolored areas (central wetwood cores) (Fig. 2) and at times nearly the entire cross section of the stem was discolored (Fig. 4). No merging of discolored zones occurred in water-treated trees (Fig. 3). However, the tissues produced following wounding, regardless of the treatment, were free of discoloration (Fig. 5) (17).

A barrier zone that protects wood produced after wounding has been shown for other species (14, 16, 21, 25). Such a barrier has special significance for elms as response of the cambium is the key to survival following multiple wounding during injection. Only the wood produced following injection can be depended upon to be living, functioning sapwood. However, if injections are repeated annually, parenchyma cells in the barrier zone could be killed and columns of discolored wood from several years could coalesce. Preventative treatments required only every three years (23) allow for wider barrier zones and more living sapwood to be produced between injections.

Late spring and early summer are the preferred times for preventative injections to insure best fungicide distribution and rapid wound closure (3, 5). Unfortunately, this was also the time when injection caused the most injury. However, shallow injection wounds and reducing the frequency of



Figure 3. Section from a water-treated elm showing three discrete areas of discolored wood.



Figure 4. Cross section of a TBZ-treated tree. Columns have coalesced to cover nearly the entire cross section.



Figure 5. Radial section at the injection site from a tree injected 16 months earlier. Clear sapwood (left) produced after wounding, treatment-initiated discolored wood (center), and wood internal to the treatment-initiated discolored wood (right) and be seen clearly.

injection are important for maximizing the amount of living sapwood available to a tree for uptake and distribution of fluids, storage of food reserves, and providing tissues able to respond to future injuries or infections (18).

To date benzimidazole compounds are the most effective fungicides registered for the control of Dutch elm disease, but because they alter the wound response of the elm unfavorably, they must be used with prudence.

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ABSTRACT

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It may seem a small matter. A child reads a poem, a group of children sing a song, they listen to a short speech that seems much too long. Then finally it's time. Eager hands scoop soil from a hole where they will plant a tree. Older hands hold the seedling as the youngsters pat the soil firmly into place. They give it water, then step back to admire it. It's their tree, and it will always be their tree. It may be the last Friday in April, or a day in May or December. Whatever the date, it's Arbor Day, and ideas as well as trees are planted on the holiday that looks ahead. This theme of planting for the future is apparent in the thousands of Arbor Day celebrations organized by communities and schools every year. And for many people, the act of tree planting evokes a special feeling. National Arbor Day is celebrated on the last Friday in April, but many states celebrate it at times more conducive to tree planting in their local climate. Nearly every Arbor Day celebration involves the planting of trees. In many communities, the celebration involves both a look to the future and a nod to the past. Some cities have a grove in honor of local men and women who have served in the armed forces. Most Arbor Day celebrations involve children, and this is very appropriate. Children who learn to appreciate and care for trees while they are young will probably be good stewards in later years. For teachers, Arbor Day provides a unique opportunity to emphasize environmental education and integrate it with other subjects.