

STRUCTURAL AND BIOLOGICAL EFFECTS OF TRUNK INJECTED PACLOBUTRAZOL IN YELLOW POPLAR

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Abstract. Sixty-three yellow poplar (*Liriodendron tulipifera*) trees, 5-11 in dbh, were selected in a plantation at the Martell Experimental Forest, Purdue University. Three trunk injection treatments were applied to three groups of 21 trees in June, 1989. Treatments consisted of injecting, at a pressure of 60 psi, 40 ml per injection hole of 1) Clipper 20 UL, 2) methanol with methylene blue dye (0.3% solution), or 3) water. The occurrence and duration of weeping from injection holes were evaluated. Sample trees from each treatment were harvested and the trunk dissected into both longitudinal and transverse sections 4 and 12 months after injection. The distance that wood discoloration or dye staining extended both above and below the injection sites and the volume of sapwood affected were substantially greater in trees injected with Clipper 20 UL or methanol/methylene blue than in trees injected with water. Closure of injection wounds was influenced by cambial and phloem dieback that resulted in nonuniform callus growth and incomplete closure of most wounds 12 months after treatment.

The high cost borne by the electric utility industry to trim trees that can grow into electric conductors provides a compelling incentive to slow and reduce the regrowth of these trees. Trunk injection of tree growth regulators has been proven, through several years of commercial trials, to be effective for shoot growth regulation in a variety of tree species (2). Regulation of growth appears to last at least three years and possibly more on most species. To sustain regulation of shoot growth for longer time periods however, retreatment will be required. Before retreatment guidelines can be established, an understanding of the structural and biological effects of injection is necessary.

One of the tree growth regulators currently available, paclobutrazol (the active ingredient in Clipper 20 UL), is a subapical meristem inhibitor which reduces growth by inhibiting gibberellin biosynthesis and cell elongation (5). Little research has been conducted to determine the physiological response of trees to trunk injection of the anti-gibberellin compounds. In a recent bibliography (4) citing 549 published articles on tree growth

regulators, only 54 deal with trunk injection of a variety of substances including nutrients, pesticides, and tree growth regulators. Only 14 of the articles deal with tree response to the injection wound. Information necessary to determine the implications of long term use of the anti-gibberellin tree growth regulators is limited. Hence, an experiment was designed to determine the structural and physiological response of yellow poplar or tuliptree (*Liriodendron tulipifera*) to trunk injection of paclobutrazol. This paper presents results concerning wood discoloration, wound closure, and weeping resulting from trunk injection.

Methods

Experimental trees were selected from a plantation of yellow poplar established in 1965 at the Martell Experimental Forest near the Purdue University campus in Tippecanoe County, Indiana. The study trees were plantation grown with many of them exhibiting suppressed growth increments due to crowding. Trees ranged from 50-70 ft (15-21 m) tall and 511 in (13-28 cm) dbh.

Sixty-three trees that appeared to be free of any defects in the lower bole were selected for this study. Defect free trees were necessary to determine tree response to treatment that was not complicated by previous wounds and internal defects.

Twenty-one trees were randomly assigned to each of three different treatment groups. The treatments were trunk injection with Clipper 20 UL (paclobutrazol in methanol carrier), methylene blue dye in methanol carrier (0.3% w/v solution), or water as a control. Initial injections were made June 26-27, 1989. An Arborchem Six Point injector was used with holes spaced at 6-8 in (15-20 cm) depending on the diameter of each tree. Each hole was drilled with a rechargeable electric drill at 24 in (60 cm) above the ground line with a brad

point bit, diameter 15/64 in (5.95 mm). Injection at a height of 24 in was done so treatment effects could be observed above and below the injection hole. Each hole was drilled on a horizontal plane, at a 45° angle, and to a depth of 2.5 to 3.0 in (6.4-7.6 cm). Trees from 6.4-7.5 in (16.3-19.1 cm) in diameter had three holes and trees from 7.6-9.4 in (19.2-23.9 cm) in diameter had four injection holes to maintain the 6-8 inch spacing between holes. Forty ml of each treatment was injected per hole at a pressure of 60 psi. Uptake of the injected fluids took a matter of seconds in most trees and 10 minutes in the slowest case. The pressure was turned off at the injection unit before closing the valve on the injector probe to release the pressure on the hole. The injector probe was left in the tree for one to two minutes after the injection unit was shut off to reduce the chances of backflow occurring.

Evaluations of all injection holes were made at three to five week intervals following injection to determine if they were actively exuding fluid onto the bark (Figure 1). This fluid exudation is described as weeping. An otoscope was used to examine injection holes internally and to observe wound closure. Fifteen trees, five from each treatment, each with four injection holes oriented at the four cardinal directions were selected for statistical analysis of weeping duration and directionality. The trees used for these observations that were made over a 66 week period were selected because they were not scheduled to be harvested during the study. An analysis of variance (ANOVA) was performed on the number of weeks that weeping occurred from each hole as well as the cardinal direction it faced. Differences between means were determined using Duncan's new multiple range test with significance at the 5% confidence level.

Three trees from each injection treatment were harvested October 24, 1989, four months after treatment. A second harvest of three trees from each treatment was made June 27, 1990, 12 months after treatment. Each tree was cut at ground line so that discoloration and dye staining both above and below the injection site could be examined. The main stem was cut just below the crown and the cross section surface examined.



Figure 1. Weeping from a trunk injection wound in yellow poplar several months after treatment with Clipper 20 UL.

This process was repeated down the bole until discoloration and/or dye was discovered. The lower 20 ft (6.1 m) of the trunk of each tree was retained for dissection and detailed study.

Two methods were used to dissect the samples for evaluation of discoloration, dye staining, and closure of injection wounds. Six of the sample trees, two of the three harvested from each treatment, were cut into cross-sectional disks 6 cm (2.4 in) thick except at the level of injection where a disk 12 cm (4.7 in) thick was cut. Each disk was measured to determine the average diameter of the wood inside the bark as well as the heartwood. From these two measurements the total volume of sapwood from the ground line to 20 ft was determined for each tree.

The dimensions of the discolored/dye stained areas that resulted from the injections were measured on both surfaces of each 6-cm-disk. In the methanol/methylene blue treatment, the dye prevented separation of tissues discolored due to injury from those simply stained. Therefore the term discolored will be used in the remainder of this paper to refer to visually altered tissues which resulted from all three injection treatments. Using the average of the area of discolored wood on both surfaces and the thickness of the disk, the volume of discolored wood in each disk was calculated for each injection hole. These were summed for the 20 ft length of each tree to give a total volume of discolored wood associated with each injection hole. The 12 cm-thick section at the level of injection was further dissected with a band saw to

facilitate examination of the injection wounds (Figure 2).

The remaining three trees from each harvest time, one from each treatment, were cut longitudinally. They were first cut into quarters on a circular saw to more manageable sized pieces. These were then cut into slabs approximately 3/8 in (1 cm) thick on a band saw (Figure 3).

Data for length, volume and percent of discolored wood were analyzed using analysis of variance (ANOVA) to examine the effect of treatments and harvest times. Differences between means were determined using Student-Newman-Keuls' test with significance at the 5% confidence level.

Results and Discussion

Weeping. Clipper 20 UL and methanol/methylene blue treatments had a consistently higher percentage of holes weeping at every evaluation time than the water treatment (Figure 4). Methanol, the carrier in Clipper 20 UL, is present in both of these treatments and may be the cause of increased weeping.

In a study on the effects of chemicals injected into the xylem of American chestnut (*Castanea dentata*) for control of chestnut blight, Rumbold (8) found that a 0.01 molar solution of methanol was not phytotoxic. However, methylene blue was slightly phytotoxic to the cambium near the point of injection. Rumbold used a 0.025% water solution of methylene blue whereas we used a 0.3% solution in methanol. This may be the cause for the higher percentage of holes weeping in the 1989 and early 1990 observations for the methanol/methylene blue treated trees versus those treated with Clipper 20 UL. Trees injected with water showed weeping too. However, the frequency declined markedly compared to the other treatments although it did continue through the second growing season for a small percent of holes.

Weeping occurrence also fluctuated during the year (Figure 4). It was observed during periods of full leaf, following leaf drop, and prior to leaf flush in the spring. Holes would frequently stop and then resume weeping. This was most noticeable during the first three months of the 1990 growing season.

Holes injected with Clipper 20 UL and methanol/methylene blue exhibited weeping for 44 and

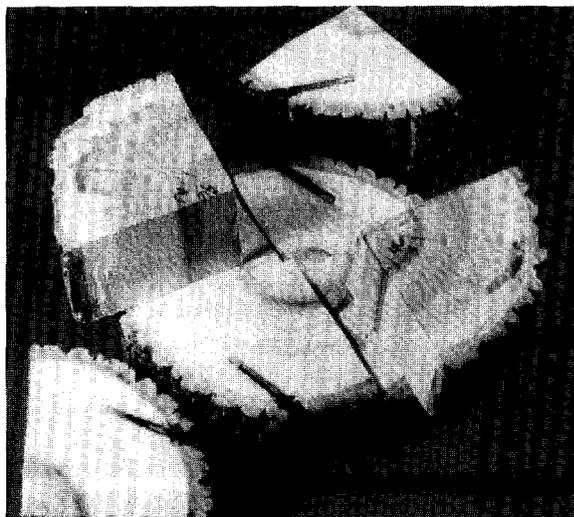


Figure 2. Exploded cross-section from yellow poplar trunk injected with Clipper 20 UL. Each injection hole was isolated and split with a band saw to reveal the wound closure pattern.

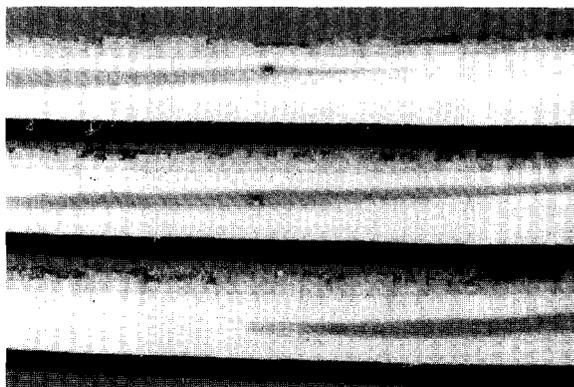


Figure 3. Longitudinal sections of yellow poplar injected with Clipper 20 UL.

46 weeks, respectively, after treatment and did not differ statistically (Figure 5). In contrast, holes injected with water exhibited weeping for only 11 weeks. There was no weeping from holes in any of the treatments in the last nine weeks of the 66 week period, ending in October 1990, during which observations were made. There was no relation between weeping and the cardinal direction the injection wound faced.

Discoloration. Dissection of trees 4 and 12 months after treatment revealed that every treatment resulted in discoloration of the xylem surrounding the injection holes. Discoloration was

present both above and below the injection site. It was frequently visible at ground line, 24 in (60 cm) below the injection site. How far discoloration extended into the root system was not determined. Length, pattern of distribution, and volume of discolored xylem were determined for the section of trunk from the ground line up to 20 feet. There was no evidence of decay in any of the trees.

The average length of discolored wood in each of the three treatments differed at both 4 and 12 months after injection (Figure 6). Discoloration was found in trees injected with water only, but the length of the affected zone was significantly less than in trees injected with Clipper 20 UL or methanol/methylene blue. Changes in the length of discoloration over time occurred only for the methanol/ methylene blue treatment. The length of discolored wood more than doubled during the eight months between the two harvest periods for this treatment (Figure 6).

All three treatments had the same discoloration pattern in the xylem near the injection site. The shape of the discolored zones when observed in the cross sections was limited to the dimensions of the injection hole initially, spread tangentially and radially with increased distance both above and below the injection holes and then tapered to a point above the injection hole and presumably below it as well (Figure 7). Clipper 20 UL and methanol/methylene blue treatments resulted in discoloration that extended below the ground line. The discoloration pattern into the root was not determined. The pattern of discoloration in water injected trees was similar but the distance it spread from the injection holes was significantly less than in the other treatments (Figure 7). These patterns of distribution also were evident in the longitudinal sections made from six trees representing one of each of the treatments at 4 and 12 months after application.

Four months after treatment, substantially more volume of discolored wood was apparent in the Clipper 20 UL and methanol/methylene blue treatments than in trees injected with water (Figure 8). The amount of discoloration did not change in trees injected with water or Clipper 20 UL during the eight months between the 4 and 12 months harvest periods. However, the volume of xylem

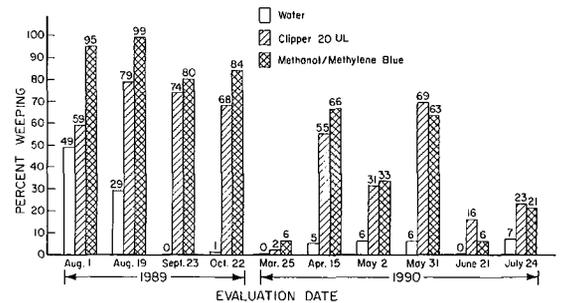


Figure 4. Percent holes weeping over time following trunk injection of yellow poplar with water, Clipper 20 UL, or methanol/methylene blue dye.

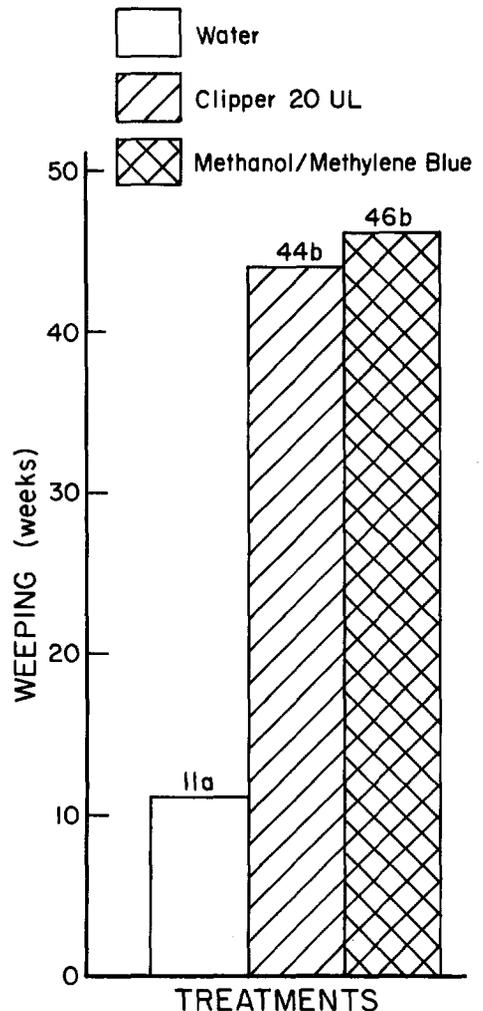


Figure 5. Duration in weeks of weeping from holes in yellow poplar following injection with water, Clipper 20 UL, or methanol/methylene blue dye. Values followed by the same letter are not significantly different at the 5% level using Duncan's new multiple range test

discolored in the methanol/methylene blue treatment almost doubled from 39 in³ (641 cm³) to 76 in³ (1253 cm³) (Figure 8).

The volume of wood visibly affected by the injection treatments initially increased with increasing distance both above and below the injection sites, but then tapered to a point at the upper end of the discolored column. For example, the average volume of discolored wood per injection hole in the 6-cm-thick disk immediately above the site where Clipper 20 UL was injected was 1.7 in³ (28.4 cm³), whereas it was 2.6 in³ (42.0 cm³) in the disk taken from 12 in (30 cm) above the injection site and 0.5 in³ (8.1 cm³) in the disk 50 in (126 cm) above the injection site. The volume expanded in both tangential and radial directions. The discoloration pattern below the injection hole followed the same general pattern as above. In water treated trees discoloration ended above the ground line, but in most of the Clipper 20 UL and methanol/methylene blue treated trees discoloration continued below ground line.

The percent volume of sapwood in the lower 20 ft (6.1 m) of the trunk that was discolored had the same pattern as the length and volume data,

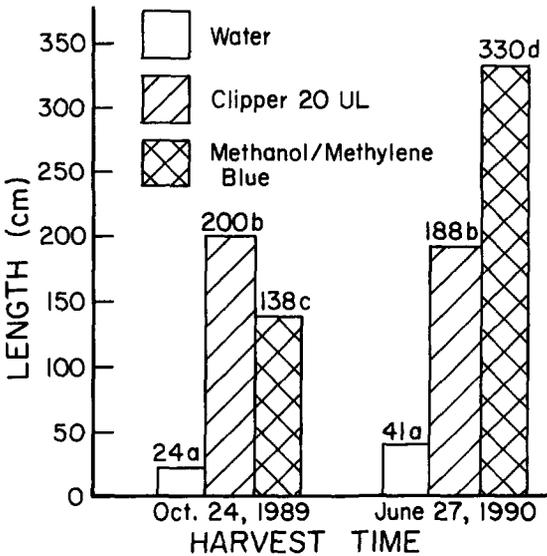


Figure 6. Length of discolored and dye stained xylem associated with injection holes in yellow poplar trees 4 and 12 months after trunk injection with water, Clipper 20 UL, or methanol/methylene blue dye. Values followed by the same letter are not significantly different at the 5% level using Student-Newman-Keuls' test.

but all three treatments were statistically the same four months after treatment (Figure 9). Approximately 3% of the total volume of sapwood was affected in the Clipper 20 UL and methanol/methylene blue treatments after four months, whereas only 0.2% was affected by water injection.

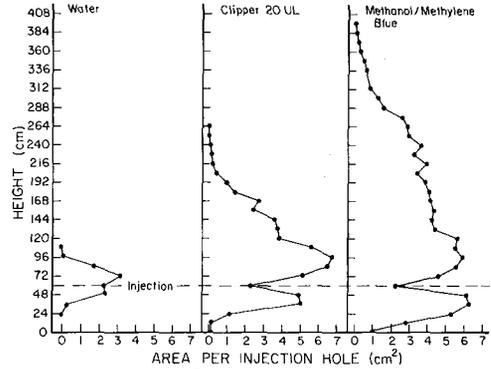


Figure 7. Area (cm²) of discolored sapwood at 12-cm-increments both above and below holes in the trunk of yellow poplar trees 12 months after injection with water, Clipper 20 UL, or methanol/methylene blue.

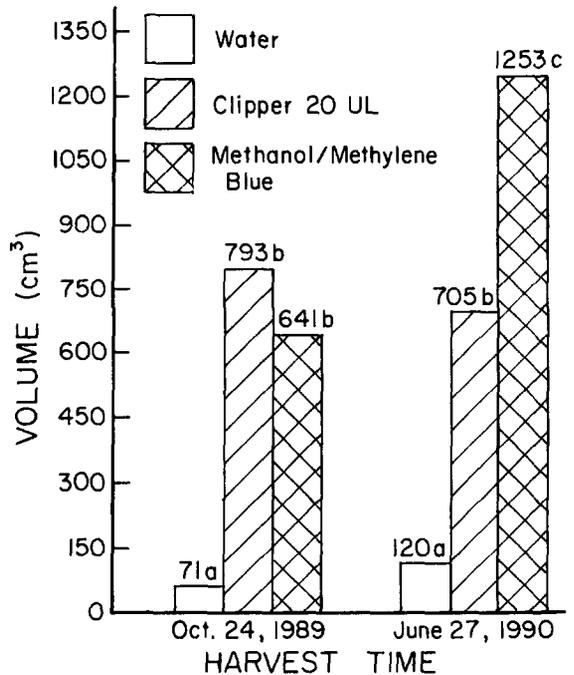


Figure 8. Mean volume per injection site of discolored and dye stained xylem associated with injection holes in yellow poplar 4 and 12 months after trunk injection with water, Clipper 20 UL, and methanol/methylene blue dye. Values followed by the same letter are not significantly different at the 5% level using Student-Newman-Keuls' test.

tion. The only change during the eight month lapse between harvest dates was a marked increase in percent discolored sapwood in the methanol/methylene blue treatment which was significantly higher than the percent discolored sapwood in water treated trees (Figure 9).

Similar patterns and occurrence of discoloration in wood associated with trunk injection have been reported for other species of trees. Shigo and Campana (9) found discolored wood associated with every hole drilled in large American elm (*Ulmus americana*) for injection to control Dutch elm disease. In another study, small American elms injected with thiabendazole plus solvent, the solvent alone, or water were dissected and evalu-

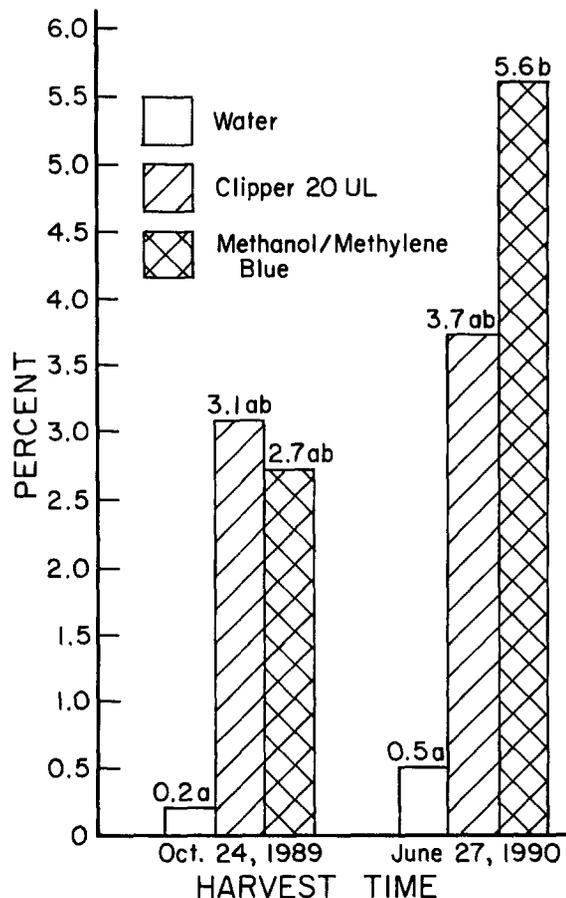


Figure 9. Mean percent volume of discolored and dye stained xylem in the lower 6.1 m of the bole of yellow poplar associated with injection wounds 4 and 12 months after treatment. Values followed by the same letter are not significantly different at the 5% level using Student-Newman-Keuls' test.

ated over a 33 month period following injection. Wounds with water injection had small columns of discolored wood while wounds with chemical injection had large columns of discoloration (3). Discoloration also was noted in the wood of sugar maple (*Acer saccharum*) both above and below the holes drilled to tap the trees for sap collection (1). Discolored wood was associated with every implantation and injection site of insecticides in white oak (*Quercus alba*) and northern red oak (*Q. rubra*). White oak trees had larger discolored areas than red oaks. Three years following treatment, about 95% of wounds had formed callus (7).

None of the treatments in the experiment reported here induced discoloration in excess of 6% of the total volume of sapwood in the lower 20 ft (6.1 m) of the trunk. For reinjection of trees, a key factor is the total circumference potentially lost due to each injection. In the case of these injections utilizing holes drilled to a depth of 2.5-3.0 in at a 45° angle and spaced 6-8 inches apart, 25 to 33% of the circumference may be lost with each injection cycle. Coalescing of injection wounds with other wounds that previously existed in the xylem as well as with the heartwood are also important factors to be considered (10). The xylem surrounding old wounds and heartwood is similar in that it is often low in stored starch which allows for greater coalescing of wounds (10). This was observed in trees in this study where injection holes were made in close proximity to the heartwood or old internal wound zones and the two columns coalesced to form one large column.

Wound Closure. Closure of the injection holes was observed periodically with an otoscope after the initial injection in June, 1989. Neely (6) describes closure of wounds as the production, differentiation, and maturation of callus parenchyma. Callus was evident within seven weeks of injection in the study trees. The callus formed at the cambial zone and closed like two sliding doors from both sides of holes. There was no evidence of callus formation from the top or bottom of holes. Within four months of injection, many holes appeared to be closed, but continued to weep. Dissection of each injection hole with a band saw revealed that the callus had grown and curled into the hole, preventing fusion of the callus tissue from each side due to bark

formation. Due to the angled injection hole the two callus masses were not directly in line with each other, further complicating closure. Most of the holes which appeared to be closed during external evaluations with the otoscope had a narrow gap where the callus had not yet fused, therefore allowing fluid to exit the hole onto the bark and prolong weeping (Figure 10). The blue colored fluid weeping from holes injected with methanol/methylene blue indicated that at least one source of the exuding fluid was from the xylem tissue into which the dye was injected.

Dissection of injection holes revealed that closure was dependent on the vigor of the tree. Trees which had produced large growth increments in the past formed more callus than suppressed trees. This observation is supported by Neely (6) who found that the amount of callus produced is directly correlated with the amount of radial stem growth at the wound site.

Dissection of each injection hole also revealed cambial and phloem dieback in 100% of the 39 holes examined. Cambial dieback at the injection site appeared consistently on the wide angle side of the hole that the drill contacted first during the drilling process (Figure 10A). The opposite side of the hole usually exhibited no cambial dieback, and this allowed for rapid growth of newly formed callus tissue into the hole. The side with cambial dieback had to form new tissue to extend across the dead cambium. Callus grew across the damaged area and then entered the injection hole. Since callus on the opposite side had already developed and grown into the hole, the two masses did not converge and fuse in a uniform fashion. Bark formation, predominantly on the callus that grew into the hole first, interfered with fusion of the two callus masses and a seam with a narrow gap resulted. This phenomenon occurred consistently in trees in all three treatments.

Phloem dieback also occurred consistently in all three treatments on the side opposite the cambial dieback (Figure 10B). Why phloem and cambial dieback occurred on different sides of the holes is not evident. It could be caused by differential effects of the physical damage induced by the force fit injection tip, the drilling process at the 45° angle, the pressurized injection using

CO₂, or a combination of these.

Summary

Fluctuations in the percent of injection holes weeping were observed for all three treatments over a 13 month period, particularly during the first three months of 1990 (Figure 4). Injection with water resulted in a lower percent of holes weeping than injection with either Clipper 20 UL or methanol/methylene blue. The methanol/methylene blue treatment had a high percentage of holes weeping during 1989 compared to the other two treatments. This response may have been due to the possible phytotoxic effects of the methylene blue dye (8) and compounded by the effects of methanol. The length, volume and percent of discolored and dye stained xylem associated with injection of trees with methanol/methylene blue almost doubled during the eight month period from 4 to 12 months after injection. Clipper 20 UL and water treated trees did not show this type of change during the same period of time (Figures 6, 8 and 9). Closure of injection holes seemed to be affected by the drilling process that caused cambial damage on one side and phloem damage on the other side of each hole. The resultant nonuniform callus growth from each side of the holes and bark formation on the callus restricted fusion and hole closure. The lack of fusion left a narrow seam between the callus masses that allowed prolonged weeping.

A second phase of this study has been initiated to determine the anatomical and physiological response of yellow poplar trees reinjected with the same materials one year after the initial injection.

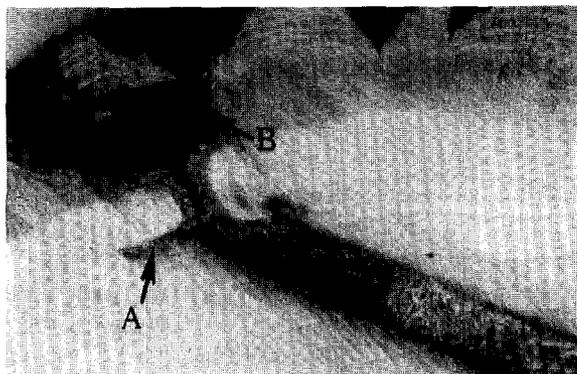


Figure 10. Cross-section of yellow poplar showing growth of callus tissue at the site of the injection hole.

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The long-awaited revision of the US Department of Agriculture's hardiness zone map is complete _ and it reveals some significant changes in North American weather patterns. The biggest alteration is a general southward drift of zone boundary lines, particularly in the Southeast and the Midwest. During the past 15 years or so, observations of local weather patterns made it clear to nurserymen and gardeners that the map's zone boundaries did not match reality. Thus many plants' hardiness zone classifications are no longer considered valid. A map revision was clearly needed. The new map is based on massive amounts of validated meteorological data from almost 8,000 government stations in the US, Canada and Mexico. The staff of Meteorological Evaluation Services was responsible for sorting through millions and millions of data pieces. The new map retains the familiar 10 zones, each of which represents an area of winter hardiness. Zones are separated by 10° increments. Each zone's temperature parameters are unchanged. In addition, the map introduces Zone 11, representing areas with average annual minimum temperatures above 40°.