HOLE ANGLE FOR TRUNK INJECTION OF TREE GROWTH REGULATORS AND ITS EFFECT ON WEEPING, WOUND CLOSURE AND WOOD DISCOLORATION

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Abstract. The effects of gibberellin synthesis inhibitors injected into tree trunks on wound exudation, wound closure, and wood discoloration were determined at various times up to 23 months after treatment. Plantation grown *Liriodendron tulipifera* trees located in Tippecanoe County, Indiana received four treatments; injection of Cutless TP or Clipper 20 UL in holes drilled at an angle (45°) or straight into the tree. A special injector tip was created for the straight injections. Injection holes drilled at 45° began weeping sooner and tended to have a higher percentage of weeping during nondormancy periods than those drilled straight into trees. Holes injected with Cutless began weeping sooner and a higher percentage wept than holes injected with Clipper. Less than one percent of the total volume of wood in tree trunks was discolored by injection treatment. The amount of wood discolored was not influenced by the compass orientation of the injection hole and was the same for treatment with either Clipper or Cutless. Straight-in injections caused more discolored wood than angle injections only in trees sampled 12 months after treatment. No difference was found in wound closure rates due to the different tree growth regulators or the angle of injection. The only benefit of making injection wounds straight into trees was a slight reduction in weeping.

Ever since electrical distribution began in the late 1800's, the demand for uninterrupted service has been increasing (1,2). Many electrical utilities report tree-related problems as a frequent or primary cause of power interruptions (18). Because trees regrow rapidly, they generally require trimming every two to five years to maintain line clearance. Any practice that reduces the volume of biomass to be removed or that extends the time period between trim cycles will save millions of dollars annually. Tree growth regulators (TGRs) reduce shoot growth and have the potential to extend trimming cycles by two to four years (1,5).

The application method for TGRs discussed in this paper is pressurized trunk injection, the principal method for application since its endorsement by the Electric Power Research Institute in 1974 (13). The advantages of pressurized trunk injection are the use of small amounts of chemical and low nontarget contact. The disadvantages discourage wider usage by utility companies. Exudation (or weeping) from injection holes is unsightly and can become a medium for growth of microorganisms. The wound itself can become a port of infection. A column of discolored wood which forms in response to the injection process may lead to decay and may possibly weaken the tree trunk.

Clipper 20 UL and Cutless TP, the leading regulators labeled for trunk injection, are subapical meristematic inhibitors. They interfere with the synthesis of gibberellins, a group of natural hormones. An advantage of subapical meristematic inhibitors is that internodes are shortened without seriously disrupting other growth processes (3).

Holes for trunk injection are currently drilled at a 45° angle. Problems associated with angled trunk injections, such as blow outs, uneven wound closure, and the difficulty of consistently drilling at a 45° angle, gave rise to the idea of injecting at a 90° angle or straight into the tree. With an injection hole drilled at a 45° angle, more cambial dieback is found on the wide angle side of the wound and more phloem damage found on the opposite side (19). Uneven damage leads to uneven wound closure, resulting in a residual seam created by bark that impedes fusion of callus tissues as the sides of the injection wound grow towards one another and meet. This residual seam may extend the time during which the wound is open for insects and pathogens to enter the tree and for exudation from the wound (19).

Another possible source of damage with angle injections is the force required to seal the standard injection probe in the hole in the tree trunk. The seal of the standard probe is created as the tapered metal tip is pushed or pounded into the injection hole (Figure 1A). Costonis (4) found a displacement of tissues by compressing action with the insertion technique used in Mauget injections.

A modified probe for straight injection was created at Purdue University to minimize the stress on tissues surrounding injection holes (Figure 1B). This probe has a section of rubber tubing near its tip. When the arms of the probe are clasped together, a metal sleeve is pushed up against the tubing, forcing it to expand, and creating a seal within the injection hole. The expanded tubing should put less stress on the surrounding tissue than the force-fit seal of the standard tip. Drilling straight into the tree should alleviate the uneven damage created as holes are drilled at an angle. This study was designed to determine 1) if the angle of injection or 2) the particular TGR injected have different effects on weeping, wound closure, or internal wood discoloration.

Materials and Methods

Thirty six trees ranging from 15 to 18 cm (6-7 inches) dbh, and 16 and 20 m (45-55 feet) high were chosen for the study from a plantation of yellow poplar (*Liriodendron tulipifera*) that were

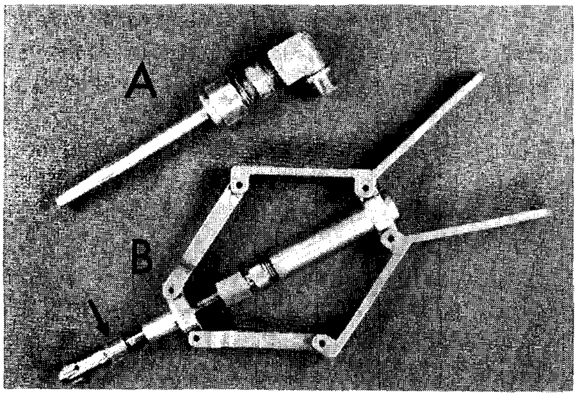

Figure 1. Standard tip with tapered shaft for angled injection (A) and tip created for straight-in injection (B). Expandable rubber tubing which creates the seal in the straight hole indicated by arrow.

established in 1965 at the Martell Experimental Forest located near Purdue University in Tippecanoe County, Indiana. Plantation trees rather than trees growing in urban areas under electric distribution lines were used to minimize the preexistence of internal defects which may have complicated tree responses to treatment and data collection.

All trees were injected July 1, 1991, at the four cardinal directions with an Arborchem Six Point injector. Trees were injected 1 m (3 feet) above ground to enable examination of effects above and below the injection sites without the need for root excavation. Injection holes were drilled 5 cm (2 inches) into the trunk with a 5/16 inch brad point bit. A 2 x 2 factorial experiment was established consisting of two injection angles, 45° and 90°, and two TGRs, Clipper 20 UL (active ingredient paclobutrazol) and Cutless TP (active ingredient flurprimidol). Forty ml of TGR were injected into each hole at 60 psi. Each tree received all four treatments.

Trees were examined for the presence or absence of exudation from each injection hole every week after treatment. Data were examined for seasonal patterns and number of days to initial weeping.

To determine the extent of wood discoloration, six trees were randomly selected and cut at the ground line at each harvest 4, 12, and 16 months after treatment. The trunks were cut into 12-cm disks until areas of discoloration in the wood, caused by injection treatment, were no longer detected. The dimensions of the discolored zones on the top and bottom surface of each disk were measured. Using these two areas of discoloration and the 12-cm thickness of each disk, the discoloration was projected through the disk to give a total volume for each disk. Volumes of discolored wood from each disk were summed to obtain the total volume of discoloration associated with each injection site (i.e. treatment). The diameter of each disk also was determined and the total volume of wood (sapwood and heartwood combined) calculated. The volume of discolored wood was expressed as a percentage of the total volume. The longest column of discolored wood was shorter than eight meters so this volume was used as the standard height to compare total and discolored wood volumes among treatments.

Because there is uneven wound closure of injection holes drilled at an angle, it was concluded to be impossible to take direct measurements of closure. Hence, wound closure was quantified via hydraulic conductivity, measured as the volume of water per unit time passing through the injection wound. The 12-cm thick section of each bole containing all four injection sites was cut with a band saw into four individual blocks each containing an injection site. The cut was made so that the interior side of each injection hole was perpendicular to the cut surface. A piece of tygon tubing was attached to the cut surface (covering the injection hole) with epoxy to form a water tight seal. Each wood block was then placed on a ring stand with the bark end of the hole facing downward towards the graduated cylinder and the tygon tubing attached to the water reservoir (Figure 2). The rate of water flow through each injection hole (i.e. treatment) was measured.

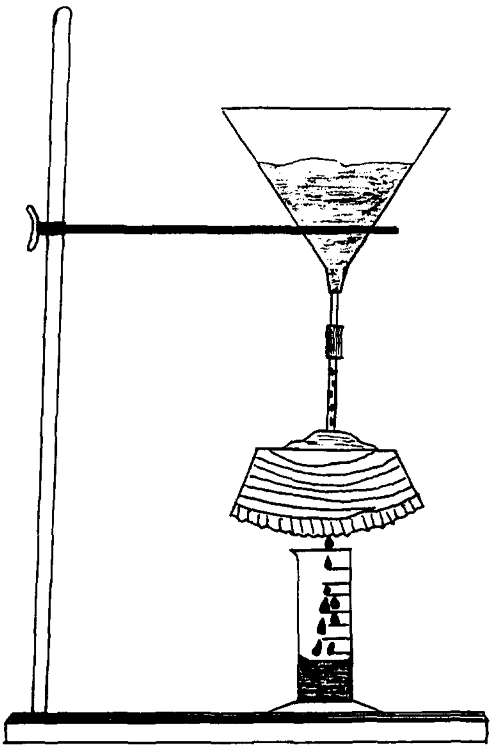

Figure 2. Funnel apparatus with attached wood block used for determination of hydraulic conductivity of holes drilled for trunk injection of tree growth regulators.

Six trees were harvested on the day of treatment, and a base line hydraulic conductivity rate for 100% open holes was determined. This was the basis for determining the percentage wound closure for the groups of six trees harvested 4, 12, and 16 months after treatment.

A General Linear Model (GML) was used to compare tree response to treatment, and Student-Newman-Keuls' was used to separate mean differences.

Results

Weeping. Holes injected with Cutless began weeping 13 days sooner (26 vs 39 days) ($p<.01$) than holes injected with Clipper. Holes drilled at a 45° angle began weeping 11 days sooner (27 vs 38 days) ($p<.01$) than holes drilled straight into trees. Seasonal patterns observed for 23 months after treatment consisted of weeping through summer, slowing in the fall, stopping in the winter, and resuming in the spring (Figure 3).

When the treatments were combined into angle of injection or TGR injected, the same seasonal pattern described above as well as other trends were found. A higher percentage of holes injected with Cutless wept every month during non-dormant periods, except that a slightly higher percentage of holes injected with Clipper wept in November and December, 1990, and in April, 1992. An equal percentage of holes injected with the two TGRs wept in March, 1991, and again at the beginning of the second growing season in May 1991 (Figure 4). A higher percentage of the holes drilled at an angle wept from July through September, 1990, and again from June through November, 1991. By May, 1992, 29% of the angled holes and only 4% of the straight-in holes were weeping (Figure 5).

Discoloration. Dissection of trees 4, 12, and 16 months after treatment revealed columns of discolored wood associated with every injection wound. These columns of wood extended above and below each injection site, tapering in both directions as they extended farther away from the wound. Total trunk volume in the first eight meters ranged from 72,200, to 189,500 cm^3, and discol-

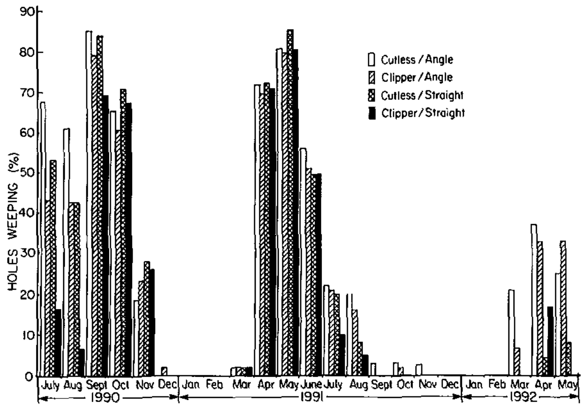

Figure 3. Percentage of trunk injection wounds weeping during 23 months following treatment with two growth regulators injected at two different angles.

ored wood accounted for only 63 to 2,920 cm^3. The amount of discolored wood averaged less than one percent of the total trunk volume. No decay was associated with the zones of discolored wood.

Within a harvest or between harvests dates there was no difference in percentage of discolored wood associated with the four cardinal directions at which injection holes were oriented. The percentage of discolored wood caused by injection with Cutless or Clipper also was not different (Table 1).

Twelve months after treatment, 0.32% of the wood was discolored in the first eight meters of the

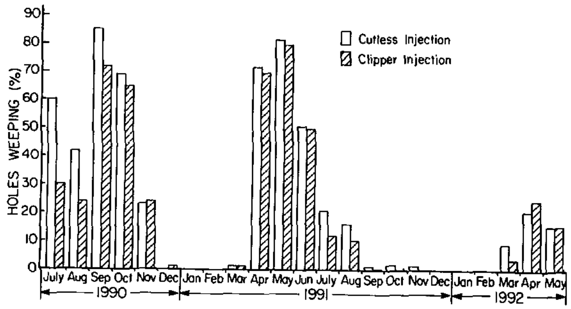

Figure 4. Percentage of trunk injection wounds weeping during 23 months following treatment with Cutless or Clipper at 45° and 90° angles.

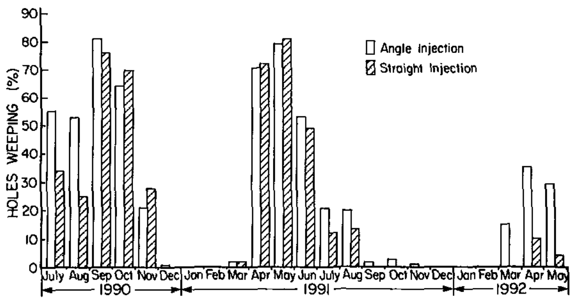

Figure 5. Percentage of trunk injection wounds weeping during 23 months following treatment with Cutless and Clipper at 45° and 90° angles.

tree trunk in association with holes drilled at an angle. This was significantly less ($p<.01$) than the 0.56% discolored wood associated with holes drilled straight into trees. No difference was found in percentage discoloration related to injection angle 4 or 16 months after treatment or between the three harvests for either angle of injection (Table 1).

Wound Closure. Closure of wounds drilled at an angle was not uniform. More cambial dieback was found on the wide angle side of the wound and more phloem damage was found on the opposing side (Figure 6). This differential damage

Table 1. Percentage of discolored wood in the first eight meters of *Liriodendron tulipifera* trunks 4, 12, and 16 months after injection on July 1, 1991 with Clipper or Cutless at 45° and 90° angles.

	Months after treatment 4	12	16
	% discolored wood		
Growth regulator			
Clipper	0.36 a#[1]	0.41 a#	0.54a#
Cutless	0.47 a#	0.50 a#	0.70 a#
Angle of injection			
45°	0.38 a#	0.32 a#	0.46 a#
90°	0.44 a#	0.56 b#	0.78 a#

1Values in columns followed by the same letter and values in rows followed by the same symbol are not statistically different at 5% using Student-Newman-Keuls'.

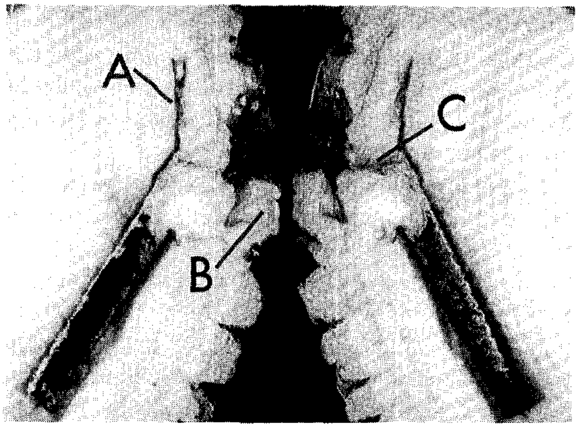

Figure 6. Cross section of *L. tulipifera* showing *growth of callus tissue at the site of the injection* hole. (A) Cambial dieback. (B) Phloem dieback. (C) Residual seam.

resulted in uneven growth of callus and allowed for incurling on one side, causing a residual seam of bark tissue that impeded fusion of callus tissues (Figure 6). Closure of holes drilled straight into trees was uniform due to an even pattern of damage to phloem and cambium around the wound (Figure 7).

The hydraulic conductivity of injection holes showed that wound closure occurred over time but that the rate did not differ between the 45° and 90° angle injection within any of the three harvests. The percentage wound closure after 16 months was significantly higher ($p<.01$) than the percentage closure found 4 and 12 months after treatment (Table 2). Wound closure rates were similar within harvest dates for treatments with Clipper or Cutless (Table 2). However, 16 months after treatment, holes injected with Clipper had significantly more closure ($p<.01$) than at 4 and 12 months after treatment. Holes treated with Cutless were progressively smaller at each harvest ($p<.01$) (Table 2).

There was no statistical difference in the rate of closure associated with the directional orientation of the injection hole within a harvest. However, progressive closure was found over time for holes facing north, east, and west. South facing holes tended to show a delay in the rate of closure (Table 3).

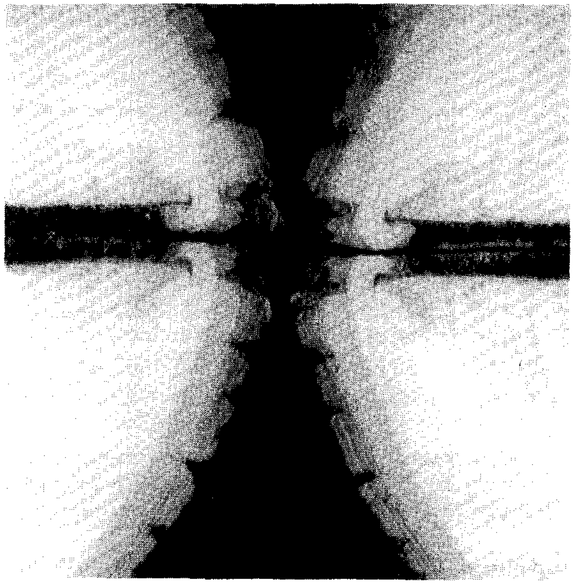

Figure 7. Cross-section view of an injection hole drilled straight into *L. tulipifera* showing uniform *damage and wound closure.*

Discussion

The back flow of the alcohol carrier from some holes immediately after trunk injection lightened the color of the bark, and the gradual weeping of tree sap from injection wounds often was colonized by microorganisms which darkened the color of the bark. Not all holes produced exudate during

Table 2. Percentage wound closure of holes in *Liriodendron tulipifera* drilled at 45° and 90° for trunk injection of Cutless and Clipper 4, 12, and 16 months after treatment on July 1, 1991.

	Months after injection 4	12	16
	% of closure		
Angle of injection			
45°	53 a#[1]	67 a#	95 b+
90°	55 a#	64 a#	92 b+
Growth regulator			
Clipper	59 a#	62 a#	92 a+
Cutless	49 a#	70 a+	95 a@

1Values in columns with the same letter and values in rows *followed by the same symbols are not statistically different* at 1% using Students-Newman-Keuls'.

Table 3. Percentage closure 4, 12, and 16 months after treatment July 1, 1991 of holes oriented at the four cardinal directions in *Liriodendron tulipifera* trunks and injected with Clipper and Cutless at both 45° and 90° angles.

Injection hole orientation	Months after treatment 4	12	16
	% closure		
North	53 a#1	73 a#+	96 a+
East	46 a#	73 a+	92 a+
South	52 a#	50 a#	85 a#
West	65 a#	68 a#	100 a+

1Values in columns with the same letter and values in rows followed by the same symbols are not statistically different at 1% using Students-Newman-Keuls'.

the first season after treatment. Sometimes one or more holes from the same tree stopped weeping and then started again with the fluctuation continuing throughout the study. This is consistent with the findings of Zillmer *et al.* (19) who examined weeping from wounds drilled at a 45° angle for trunk injections of Clipper, water, and methylene blue dye in yellow poplar trees.

Holes drilled at an angle wept significantly sooner and had a higher percentage of weeping in 75% of the months during non-dormancy periods than holes drilled straight into the trees. This may have been caused by a higher degree of damage to phloem and xylem tissues by drilling at a 45° angle. Holes drilled at an angle consistently led to more cambial dieback on the wide angle side of the wound and more phloem damage on the opposite side (Figure 6). A similar pattern of phloem and cambial damage was reported by Zillmer *et al.* (19) for injection wounds drilled at 45°. In an angled injection, the drill bit contacts the wide side of the injection hole first and rests on that side as the hole is drilled. This uneven damage was not found with straight injections (Figure 7).

At the beginning of the second growing season after treatment, May, 1992, a higher percentage of holes drilled at an angle than straight into the tree were weeping. Because of uneven damage during drilling, a residual seam was created as angled holes closed (Figure 6). This seam may be the reason for the extended time during which the wound is open to release exudate. Because holes drilled straight-in were evenly damaged during the drilling process, the two sides of callus were better matched for fusion of xylem and phloem tissues as they grew together.

Holes injected with Cutless began weeping sooner and had a higher percentage weeping in 88% of the months than those injected with Clipper. This may have occurred because of different toxicities of the TGR carriers. Isopropanol is the carrier for Cutless, and methanol is the carrier for Clipper. Since the active ingredients (flurprimidol for Cutless and paclobutrazol for Clipper) in the TGRs make up less than 3% of the solution injected, and their physiological activity is similar, it is unlikely that they are the source of the difference.

Seasonal weeping patterns, consisting of weeping during summer, slowing down in autumn, stopping during winter, and starting again in spring, were expected because they follow the physiological dormancies and activities of the trees.

Wood discoloration is a response to wounds caused by injections, pruning, tapping for sap, increment boring, and bird and squirrel damage (6,7,9,15,16,17). Therefore, the discolored wood found in trees in this study probably was not caused by the TGRs alone, but was more likely caused by a combination of the mechanical damage done by drilling holes, the pressure used for injection, and the chemicals injected. Both the angled and straight injections with either of the two TGRs caused less than one percent discolored wood in the first eight meters of the tree trunks. The higher percentage of discolored wood associated with the straight-in injections 12 months after treatment likely occurred because the seal of the straight injector tip was set too shallow in the hole or it did not form a tight seal and exposed xylem and phloem to the alcohol. Alcohol carriers contacting cambial tissue can damage cells surrounding injection sites or result in cambial dieback (8,11).

Wounds close by the production, differentiation, and maturation of callus parenchyma (12). Gibbs and Smith (6), McQuilken (10), Neely (12), and Rumbold (14) agree that the production of callus progresses toward the center of a wound until the opening is closed. Very little callus tissue was produced from the top and bottom of each wound in this study.

It was anticipated that straight-in injection holes would close faster than angled injection holes because of the uneven wound closure (Figure 6) of holes drilled at an angle. However, no difference was found (Table 3). This may also be related to the shallow seal or lack of a seal for the straight injection. Since the straight injections had more tissue exposed to alcohol, a larger wound was created, more damage probably resulted, and the wounds closed slower.

Summary

Results of this study do not provide a basis for strong endorsement of making injections straight into trees or for the tip constructed to make this type of injection. The enhanced wood discoloration and damage noted in some cases with the straight-in injection may have been caused by poor positioning and sealing of the end of the injector tip. Since this may have been the only problem, further research with shallow, straight-in injection seems warranted.

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Résumé. Les effets des composés-synthèse de gibbérelline, employés comme inhibiteur de croissance, sur l'exsudation des blessures, la cicatrisation et la coloration du bois, lors d'injections dans les troncs d'arbres, étaient évalués jusqu'à 23 mois après le traitement, et ce, au cours de diverses périodes. Les *Liriodendron tulipifera* d'une plantation reçurent quatre traitements: une injection de Cutless TP ou de Clipper 20 UL par des trous forés à un angle de 45° ou à 90°. Les trous d'injection forés à un angle de 45 commençaient à couler peu de temps après et tendaient à présenter un pourcentage plus élevé d'écoulement lors des périodes de végétation que ceux forés à angle droit. Les trous injectés avec le Cutless commençaient à couler plus tôt et à un nombre plus élevé que ceux avec le Clipper. Moins de un pourcent du volume total de bous du tronc était coloré à la suite de l'injection. Les trous à angle droit produisaient plus de coloration dans le bois que ceux à 45°, mais seulement sur les arbres échantillonés 12 mois après le traitement. Aucune différence était observée sur le processus de cicatrisation, que ce soit en rapport avec le type de produit utilisé ou l'angle de forage des trous d'injection.

Zusammenfassung. Die Auswirkung des Gibberellin synthesehemmers, der in die Baumstämme injiziert wird, wurde auf Überwallung und Holzverfärbung zu verschiedenen Zeiten nach der Behandlung - bis zu 23 Monate - bestimmt. In Plantagen gewachsene Bäume (*Liriodendron tulipifera*) erhielten vier Behandlungen; Cutless TP oder Clipper 20 UL wurden in den Baum injiziert in Löcher, die mit einem Winkel von 45° gebohrt wurden. Injektionslöcher, die auf 45° gebohrt wurden, leckten eher und hatten tendenziell einen höheren Prozentsatz an Leckstellen während der Vegetationszeit als die, die gerade in Bäume gebohrt wurden. Löcher, die mit Cutless injiziert wurden, fingen eher zu nässen an und hatten zudem einen höheren Prozentsatz an Leckstellen als die, die mit Clipper injiziert wurden. Weniger als ein Prozent des Gesmtvolumens des Holzes in den Baumstämmen wurde durch die Injektionsbehandlung verfärbt. Injektionen, die gerade in die Bäume erfolgten, verursachten mehr Holzverfärbungen als Winkelinjektionen, allerdings nur in Bäumen, die 12 Monate nach Behandlung untersucht wurden. Die unterschiedlichen Wachstumsregler oder der Winkel der Injektion verursachten keine Unterschiede bei der Überwallungsrate.