Jour. Korean For. Soc. Vol. 99, No. 2, pp. 220~225 (2010)

JOURNAL OF KOREAN FOREST SOCIETY

Effects of Wound Dressing with Thiophanate-Methyl Paste on Compartmentalization of Pruning Wounds

Kyu Hwa Lee* and Kyung Joon Lee

Department of Forest Sciences, Seoul National University, Seoul 151-921 Korea

Abstract : This study was conducted to examine the effects of wound dressing with thiophanate-methyl paste on the compartmentalization of pruning wounds in *Acer palmatum*. A total of thirty field-grown trees were used for three different treatments, such as no dressing, dressing once right after pruning cut, and dressing twice, one more dressing treatment one year after initial dressing. Wound closure rate (WCR) and discolored/wound area ratio (D/W ratio) two years after treatment were measured. Variations of extractives, holocellulose and lignin at the treated branch unions were also examined. The WCR of no dressing treatment of 70.9% was significantly lower than those of the two dressing treatments (85.4% and 82.7%, respectively), while the difference between dressing once and twice was not significant. The D/W ratio of no dressing treatment (39.3%) was significantly higher than those of the two dressing treatments (around 30%). Generally, at the branch core of the treated union, contents of extractives and lignin were higher and holocellulose contents were lower than the branch core of the union with living branch. Among the branch core of treated union, no dressing treatment showed a relatively lower holocellulose (63.5%), and relatively higher extractives (2.8%) and lignin (26.6%) than dressing once (66.2%, 1.7%, 26.1%, respectively).

Key words **:** *Acer palmatum, wound closure, extractives, holocellulose, lignin, discolored area*

Introduction

Constant efforts were made to find an ideal wound dressing which should prevent decay, stimulate wound closure, and improve tree appearance, but to date no dressing commercially available has been shown to prevent decay (Dujesiefken *et al*., 2005; Gilman, 2002; Harris *et al*., 2004; Schwarze *et al*., 2000; Shigo, 1986).

Shigo and Wilson (1977) applied asphalt, polyurethane, and shellac to protect wounds on red maple and American elm, and found that none affected vertical extensions of the discolored and decayed wood or the presence of decay fungi. Various wound dressings ranging from commercial tree paints, orange shellac, asphalt paints, creosote paints, and house paint to grafting wax were advised to use over the years. But current research indicates that these materials are not effective in protecting trees from wood-rotting organisms (Hartman *et al*., 2000).

A few effective results were reported from the experiments with plastic wrap (Shortle and Shigo, 1978) and some plant growth regulators (Ha, 2004; Hartman *et al*., 2000). But most authors do not recommend wound dressing (Gilman, 2002; Harris *et al*., 2004; Hartman *et al*., 2000), and American National Standards Institute (2001) even prescribes that wound treatments should not be used to cover wounds or pruning cuts, except when recommended for disease, insect, mistletoe, sprout control, or for cosmetic reasons.

Regardless of those prevailing recommendations, applying thiophanate-methyl paste (Topsin Paste^{TM}) on the tree wounds is generalized practice in Korea without any experimental tests. This study was conducted to examine the effect of wound dressing with the thiophanate-methyl paste on the compartmentalization of pruning wounds. The tree species selected for this experiment was *Acer palmatum* which is broadly planted in the urban forest of Korea.

Materials and Methods

1. Tree species for the study

Thirty field-grown *A. palmatum* (Japanese maple) trees, which were growing in Yeoju, Gyeonggi, Korea (37.10.841 N, 127.38.900 E), were used for this study. The trees were 13-year-old, 10.3 cm thick in average diameter at 30 cm above the ground, and 360 cm tall in

*Corresponding author
E-mail: qhalee53@snu.ac.kr

Table 1. General description of experimental trees (*Acer palmatum*) and experimental conditions; average trunk diameter at 30 cm above ground in April 2007, and average diameter of pruned branch by treatment. Means with the same letter are not significantly different at P<0.05.

unit: cm

Trunk diameter	Diameters of pruned branches		
	No dressing	Dressing once	Dressing twice
10.3 ± 0.1*	1.94 ± 0.07 a	1.98 ± 0.08 a	2.00 ± 0.07 a

*Mean ± standard error

average height in April 2007 (Table 1).

2. Experimental design

The thirty trees were allocated according to the randomized block design. The stand of the trees was divided into three blocks, which were border, intermediate between border and interior, and interior of the stand. Each block of the treatments consisted of ten trees.

3. Treatment

Pruning wounds were treated by three different ways, which were 'no dressing' as a control in treatments, 'dressing once', dressing right after pruning cut, and 'dressing twice', one more dressing treatment one year after initial dressing. The sizes of the branches for pruning cuts were between 1 cm and 4 cm in diameter at 1.5 cm above the branch bark ridge (BBR). The number of removed branches per tree was multiples of three, depending on the branch density of the tree, to allocate the same number of wounds with similar sizes by treatment. Consequently, average diameters of pruned branches by treatment were not significantly different (Table 1).

The pruning wounds were dressed with thiophanate-methyl paste (Topsin Paste^{TM}, a product of Nippon Soda Co., Ltd.). Pruning and wound dressing treatments were made in mid-summer (August 17, 2007) before most decay fungi were sporulating in the fall (Hartman *et al*., 2000; Shigo, 1989). All the treated trees were harvested two years after initial treatments.

4. Pruning cuts

Pruning cuts were conformable with the American National Standard (American National Standard Institute, 2001). A pruning cut was made close to the trunk or parent limb, without cutting into the BBR or collar, or leaving a stub (Figure 1). In case where branch collar was not readily apparent, the cut bisected the angle between its BBR and an imaginary line perpendicular to the branch.

5. Measurements

1) Branch and stem diameter and area of pruning wound

prior to pruning, diameters of branches and stems at

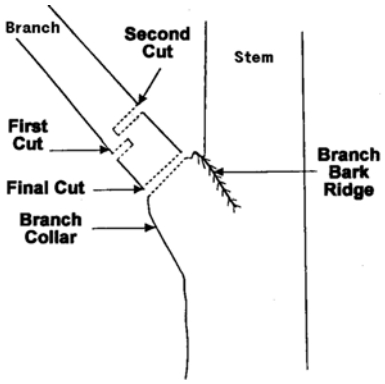

Figure 1. A pruning cut was made close to the stem without cutting into the branch bark ridge, or leaving a stub. A branch too large to support with one hand was precut to avoid splitting of the wood or tearing of the bark (American National Standard Institute, 2001).

1.5 cm above the BBR were gauged with a caliper. Immediately after each cutting, horizontal width and vertical length of the pruning wound crossing the center of the cut branch were measured to estimate the wound area.

2) Discolored area

The harvested samples were dissected with a cleaver along the medial longitudinal plane and shaved with a sharpened knife to measure discolored areas formed after the branch removal. The area of discolored stem tissue on the medial longitudinal surface was estimated by the length of pruning wound (B-B') and the deepest penetration of discolored wood into the trunk (H-H') with the formula squaring the area of a triangle supposing the

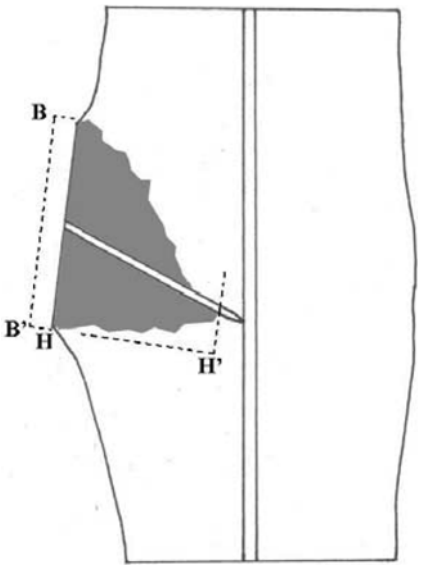

Figure 2. A diagram of a branch union sample split along the medial longitudinal plane one year after pruning cut. Length of pruning wound (B to B') and depth of the discolored wood (H to H') were used for measuring the area of discolored wood inside the pruning wound.

shape of discolored wood as a triangle (Figure 2). The length of H-H' was measured along the imaginary line perpendicular to the pruning wound.

6. Determinations of extractives, holocellulose and lignin

Chemical analyses for the tissues of the branch core, and the above and below core were performed to examine the changes of their chemical properties two years after treatments. Two trees from each treatment were selected for the analyses. A total of sixteen branch union samples, eight from each tree, were collected from each treatment. The eight samples of each tree consisted of four with pruning wounds and the other four with living branches.

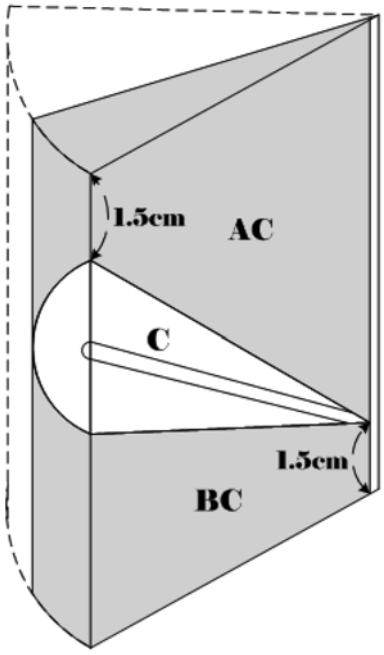

Figure 3. A diagram of a branch union sample dissected along the medial longitudinal plane. The parts beyond an imaginary line perpendicular to the stem pith at 1.5 cm above the branch bark ridge and an imaginary line perpendicular to the stem pith at 1.5 cm below the end of branch pith were trimmed off. The trimmed sample was divided into three parts, the core of branch (C), above core (AC) and below core (BC) for chemical analyses of those tissues.

The samples were dissected with a cleaver along the medial longitudinal plane, and the parts beyond an imaginary line perpendicular to the stem pith at 1.5 cm above the BBR and an imaginary line perpendicular to the stem pith at 1.5 cm below the end of branch pith were trimmed off (Figure 3). The trimmed samples were air-dried, split into three parts (core of branch, above and below core), and then ground through a 40 mesh screen.

The content of the extractives, was determined by the soxhlet method (Technical Association of Pulp and Paper Industry, 1994). Two grams of sample in a thimble filter was extracted by 150 mL of ethanol/benzene mixture (1:2 v/v) using a soxhlet extractor with reflux condenser at 80°C for 6 hours. The extracted solution was evaporated under reduced pressure. The extractive was dried at 105±1°C and then weighed. The extractive-free sample left in the thimble filter was used for analyses of holocellulose and Klason lignin.

The content of holocellulose was determined as the delignified residue by $NaClO_2$ (Wise *et al*., 1946). The 1.25 g of extractive-free sample in 250 mL flask was treated with 75 mL of distilled water, 0.5 g of $NaClO_2$ and 0.1 mL of CH_3COOH at 80C for 1 hr. This procedure was repeated 2 more times. The solution was filtered by using a glass filter (1G3, Iwaki, Japan), and then washed with 250 mL of cold distilled water and 25 mL of acetone, successively. The filtrated residue was dried at 105±1°C and then weighed.

Klason lignin was analyzed according to the standard NREL procedures (National Renewable Energy Laboratory, 2005). The 0.3 g of extractive-free sample in 50 mL flasks was hydrolyzed with 3 mL of 72% H_2SO_4 at 30°C for 1 hr. The hydrolysate was then transferred to 100 mL flask and diluted to 4% H_2SO_4 by adding 84 mL of distilled water. The flasks were sealed and autoclaved for 1 hr at 121°C. The solution was then filtered by

Table 2. Indicators to analyze the changes in the area of pruning wound, and development of discolored wood on the medial longitudinal surface two after treatments.

Indicators	Formulae
Wound Closure Rate (WCR, %)	$WCR = \frac{PWA_{y0} - PWA_{y+2}}{PWA_{y0}} \times 100$
	PWA_{y0}: area of pruning wound at the time of treatments PWA_{y+2}: area of pruning wound 2 years after treatments
Discolored/Wound Area Ratio (D/W ratio)	D/W ratio $= \frac{DA_{y+2}}{PWA_{y0}}$
	DA_{y+2}: area of discolored stem tissue on the medial longitudinal surface two years after treatments PWA_{y0}: area of pruning wound at the time of treatments

using a glass filter (1G4, Iwaki, Japan). The filtrated residue was dried at 105±1°C and then weighed.

7. Data analysis

Indicators of wound closure rate (WCR) and discolored/wound area ratio (D/W ratio) were introduced to examine the changes of abilities to compartmentalize the pruning wounds after the treatments (Table 2). The WCR represented the enclosing extent of the pruning wound during two years after treatments, and the D/W ratio was computed by comparing the area of discolored stem tissue on the medial longitudinal surface two years after treatments with the area of pruning wound at the time of pruning. In the CODIT model (Shigo and Marx, 1977), the WCR indicated vitality of the cambium around the wound which formed wall 4 after the tree was wounded, while the D/W ratio expressed the ability of a tree to limit the spread of wood discolorations and decays with wall 1, 2 and 3 which were already present in the wood at the time of the pruning.

The two-way Repeated Measures ANOVA was used to calculate the ratio of the variance among the means to the variance within the samples. Since the covariance matrix did not satisfy the sphericity, univariate tests of hypotheses for within subject effects could not be used. Instead, multivariate test was used. It provided several statistics such as Wilks' lambda, Pillais trace, Hotelling-Lawley trace and Roy's greatest root (Hair *et al*. 2006). Using these statics, *P*-value was calculated by asymptotic F distribution. The statistical difference among the means of the treatments was determined using the Duncan's multiple comparison procedure (Davis, 2007; Norman and Streiner, 1998). SAS 9.1 (SAS Institute Inc., USA) was used for the analysis, and the significance level, α-value, was 0.05 unless otherwise specified.

Results and Discussion

1. Wound closure and stem discoloration

Table 3 shows variations of the WCR and the D/W ratio by treatment two years after treatments. The WCR of no dressing treatment of 70.9% was significantly lower ($P < 0.05$) than those of the once and twice dressing treatments (85.4% and 82.7%, respectively), while the difference between dressing once and twice was not significant. This implied that the dressing after development of the woundwood was not inefficacious. On the contrary, the D/W ratio of no dressing treatment (39.3%) was significantly higher ($P < 0.01$) than those of the two dressing treatments (around 30%), and the difference between dressing once and twice was not significant, either.

Table 3. Variations of wound closure rate (WCR), and discolored/wound area ratio (D/W ratio) by treatment two years after treatments. Means with the same letter are not significantly different at $P < 0.05$.

Treatments	WCR (%)	D/W ratio (%)
No dressing	70.9 ± 3.2* a	39.3 ± 1.1 a
Dressing once	85.4 ± 2.2 b	30.7 ± 0.9 b
Dressing twice	82.7 ± 2.5 b	29.8 ± 1.3 b

*Mean ± standard error

Many studies confirmed that the use of wound sealants was not an adequate substitute for good compartmentalization and rapid wound closure (Harris *et al*., 2004; Schwarze *et al*., 2000; Shigo, 1983). Only a few researches reported effectiveness of the dressing on the wound closure. Application of a lanolin slowed drying of wounds and hastened initiation of callus growth (McQuilkin, 1950), and plant hormones have been found to be variable in their effects on the callus production (Ha, 2004; Hartman *et al*., 2000).

Topsin Paste™ is a fungicide to control a canker on the apple tree (*Valsa ceratosperma*), and its ingredients are 3% of thiophanate-methyl and 97% of a mixture of colorants, antifreezes and solvents. Considering the expressed ingredients, it is hard to discuss this result of the rapid wound closure by applying the fungicide. Further researches are necessary to determine the major components of the fungicide to promote the callus production.

Low discoloration at the treated pruning wounds might be resulted from several events. The wound dressing might protect the exposed cambium from the dieback, which could be helpful to the wound closure, and the fast closure of pruning wound would restrict the decay process of the wounds (Shigo and Larson, 1969). In addition, the applied fungicide might have slowed down the rate of decay process (Acquaah, 2002), and the paste might prevent wounds from drying up (McQuilkin, 1950).

2. Variations of wood chemical property

Figure 4 shows variations of the extractives, holocellulose and lignin in the three parts (above core, branch core and below core) among the branch unions with dressing treatments and the unions with living branches. Generally, at the branch cores of the treated unions, the contents of extractives and lignin were higher, and the contents of holocellulose were lower than those at the branch cores of the unions with living branches. Among the treated unions, no dressing treatment showed relatively lower holocellulose (63.5%), and relatively higher extractives (2.8%) and lignin (26.6%) than dressing once (66.2%, 1.7%, 26.1%, respectively).

A tree begins to form a protective chemical shield, a reaction zone, around and immediately behind the

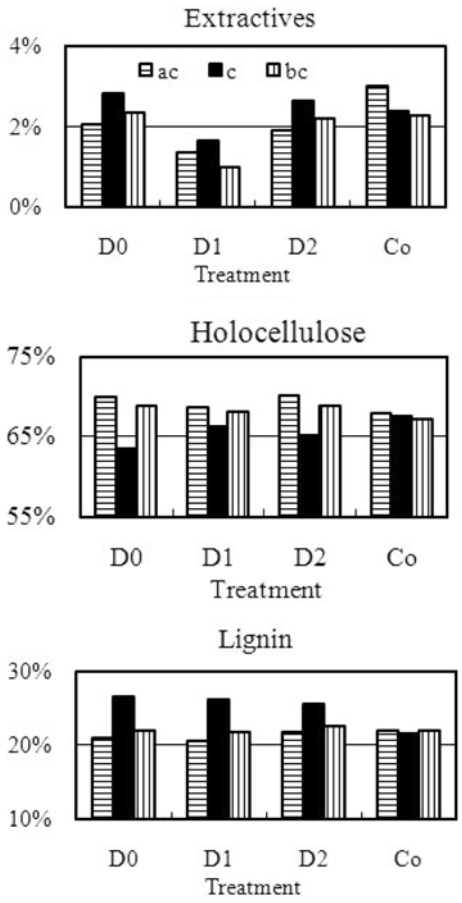

Figure 4. Variations of extractives, holocellulose and lignin in the three parts (ac: above core, c: branch core, bc: below core) by treatment. D0: no dressing, D1: dressing once, D2: dressing twice, Co: control-branch union with living branch.

wound (Shigo, 1979; Shigo and Marx, 1977). As time goes by, the xylem compromised by the wound starts to re-wet, and a region corresponding to the site of formation of the colored reaction zone becomes wetter than the adjacent healthy wood. This water may carry secondary metabolites produced by the elicited xylem parenchyma cells to developing reaction zone and would permit their accumulation in the nonliving cells of the differentiated wood (Pearce, 2000). So the branch core of pruned branch union contained more extractives than the other parts of above and below core of the union.

Formation of lignin is another defense mechanism in the wood tissues to protect trees from wounding and microbial invasion (Yamada, 2001). Geiger *et al.* (1986) reported an increase of 25-30% in lignin-like materials in the wood of *Hevea brasiliensis* taproots close to the infection front of *Rigidoporus lignosus*. Increases of lignin contents at the core of no dressing treatment by about 23% comparing with the core of the control (21.6%) in the present study would result from the lignification of exposed xylem tissues as a defense mechanism of the trees.

Conclusions

This study showed that the dressing of pruning wounds with thiophanate-methyl paste (Topsin Paste™) at the time of pruning was effective in the wound closure and slowing the discoloration of wounded tissue. However it was hard to find the cause of the effectiveness with the expressed ingredients. Further study is required to identify the effective ingredients in wound protection, which contribute to the tree health.

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(Received January 21, 2010; Accepted March 22, 2010)