Ethylene Production by Excised Sapwood of Clonal Eastern Cottonwood and the Compartmentalization and Closure of Seasonal Wounds

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ABSTRACT

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Increment cores were removed from ramets of six clones of eastern cottonwood at 3-mo intervals starting either in November, at the beginning of the dormant season, or in May, at the beginning of the growing season. By offsetting the two wounding series by 6 mo, it was possible to separate the effect of wound age from the effect of season of wounding with regard to dynamic host responses and the fungi that colonize such wounds. Production of ethylene by these cores of outer sapwood was measured 1 (et_1) and 2 (et_2) days after their collection and incubation in sealed containers under standardized conditions. Methane, a product of methogenic bacteria, was measured 1 day after core collection. Observations on wound closure were made at 3-mo intervals. At the time of harvest, each tree had wounds 3, 6, 9, and 12 mo old. Discoloration associated with wounds initiated during the growing season was significantly less than that associated with wounds initiated during the dormant season regardless of wound age. Clones differed in their capacity to compartmentalize wounds. Ethylene production (et_1) by increment cores collected in February correlated best with the ranking of mean clonal discoloration. The seasonal course of et_1, but not et_2, across clones faithfully mirrored that expected for the physiological activity of sapwood; i.e., it increased significantly through February, November, August, and May. The ratios of basal (February) to maximal (May) rates of et_1 ranged from 2.7 to 5.6 for better compartmentalizing clones and from 1.4 to 1.7 for poorer compartmentalizing clones. It is suggested that ratios of et_1 may be used to rapidly screen for superior compartmentalizing genotypes, although additional studies are necessary to confirm or refute this hypothesis. Wounds largely closed during the 3-mo period from May to August. Clones differed significantly in their rate of closure. Those with higher et_2 in May tended to close more rapidly. Wound closure and compartmentalization, however, were not significantly related, nor was methane emanation significantly related to either of these host responses. Season of wounding seemed more influential than age of wound with regard to fungal colonization. *Fusarium solani* was isolated significantly more frequently from wounds initiated during the growing season, whereas decay fungi were isolated significantly more frequently from wounds initiated during the dormant season, regardless of wound age.

Compartmentalization of infected sapwood and wound closure are two active mechanisms of defense exhibited by perennial hosts. Shigo et al (18) reported that some poplar clones differ significantly in their capacity to compartmentalize infected sapwood. Heritability estimates, furthermore, indicated that both of these defense mechanisms are under genetic control, although they were not significantly correlated (2).

An orange, phenol enriched, highly tylosed zone separating discolored/decayed wood from sound sapwood was observed in the sapwood of poplar (11,19). This reaction zone (13) may account for the localization of wound-invading microorganisms. A process to rapidly identify genotypes with greater resistance to decay/discoloration would be useful in selection/breeding programs as well as in basic studies to clarify resistance mechanisms. Such studies should take into account the effect of season on the hosts' capacity to respond to wounding and the microorganisms' capacity to colonize wounds. It was suggested that a succession of microorganisms occurs in wounds over time with non-Hymenomycetes eventually giving way to hymenomycetous decay fungi (16).

The objectives of this study were to separate the effect of season of wounding from wound age with regard to host defense and the fungi that colonize such wounds and to seek a rapid predictor of the hosts' capacity for defense. Ethylene production by outer sapwood excised at different seasons was chosen for study. Ethylene has been implicated in the mediation of processes related to host resistance (3).

A preliminary report on these studies was presented earlier (15).

MATERIALS AND METHODS

Six clones in an 11-yr-old planting of eastern cottonwood (*Populus deltoides* Bartr.) located on the flood plain of the Ohio River in Hancock County, Kentucky, were used for these studies. Four of the clones were selected originally at the Southern Hardwoods Lab, U.S. Forest Service at Stoneville, MS. These are Stoneville (St) clones 74, 75, 92, and 109. The most northern of these is St 109, which originated in Bolivar County, Mississippi. Clone Wickliffe (Wi) 1 originated in Ballard County, Kentucky, whereas clone Alton (Alt) 1 originated in Alton County, Illinois. Clones were chosen after preliminary studies to include good and poor compartmentalizers. The average height and diameter, at 1.4 m, of these trees at 10 yr of age was 20.9 m and 23.4 cm, respectively (22).

Tree wounding and harvest. Wounds were made at a height of 1.4 m to a depth of 3.0 cm with an increment borer (0.5 cm inside diameter, 1.05 cm cutting thread diameter). After their removal from the tree, cores were examined to assess the extent of discoloration, if present, at the time of wounding. Wounds then were widened slightly with a 1.27-cm bit to facilitate collection of gases from the wound at the time of wounding.

The wound and harvest schedule can be seen in Table 1. A total of 36 trees (six in each of six clones) were divided equally into two series. Series 1 consisted of trees whose oldest wound (12 mo) was initiated at the beginning of the dormant season (November 1983); Series 2 consisted of trees whose oldest wound was initiated at the beginning of the growing season (May 1984). Trees were wounded in each of the four seasons so that, at the time of harvest, each tree had a wound 12, 9, 6, and 3 mo old spaced equally around its circumference. Wound aspect was chosen to minimize the effect of solar radiation during the year; i.e., wounds on the north, east, south, and west aspects were initiated during August, May,

February, and November, respectively. Preliminary tests indicated that wounds initiated at different aspects during the same season did not differ significantly in their associated discoloration or in the amount of ethylene produced by excised increment cores. This design permitted the comparison of wounds made on the same clone during the same season but differing in wound age by 6 mo.

Gas analysis. Gases in wounds were collected to determine those present at the time of wounding. This was done by placing a stopper, fitted with tubing, in the mouth of the wound and evacuating the wound to satisfy a negative pressure of 0.5 atm in a 6-ml sealed tube.

Of particular interest was the amount of ethylene produced by each extracted increment core. Bark and cambium were removed from cores, and the outer clear xylem, to 1.5 cm in depth, was placed in a tube that then was sealed with a septum. Discolored inner portions of increment cores were excluded from analyses. Tubes with increment core segments were refrigerated on ice until their return to the laboratory where they were incubated at 25 C.

Gas analyses were conducted 1 and 2 days after collection of increment cores. One milliliter of gas was withdrawn from each tube and injected into a Varian 2700 gas chromatograph equipped with a flame ionization detector as described previously (14). Tubes were flushed with air at the end of the first-day determinations, then resealed and returned to 25 C for incubation before the second-day determinations. Increment cores then were dried at 80 C to constant weight so that the amount of gas detected could be expressed per unit dry weight of xylem.

Assessment of wound closure, compartmentalization, and fungi associated with discoloration. Wounds were observed at 3-mo intervals and rated for closure on a scale of 1 (open)–4 (closed); ratings of 2 and 3 were assigned to wounds one-third and two-thirds closed, respectively.

A stem disk about 25 cm long, i.e., from about 5 cm below to 20 cm above wounds, was cut from each tree at the time of harvest. The upper portion of each wound was split longitudinally through the wound to expose a radial surface. The proportion of this radial surface occupied by discolored wood, from the depth of the wound in formerly clear sapwood to a line 14 cm above the wound was taken as a measure of the tree's capacity to compartmentalize (Fig. 1). Poor compartmentalizers thus had a greater proportion of discolored wood associated with their wounds.

Radial wound surfaces were surface sterilized by alcohol and flame before the removal of wood chips for culture in acidified malt extract (2%) agar (2%) or benomyl (2 ppm) malt (2%) agar (5). Four chips from each wound were placed on each medium. Cultures were examined for fungi 1 and 2 wk after incubation in the dark at room temperature.

Statistical analyses, unless stated otherwise, were based on SAS procedures for analysis of variance and, when significant F values were obtained, multiple comparisons of least square means of log converted values.

RESULTS

The effect of age of wound and season of wounding on wound associated discoloration of sapwood can be seen in Table 1 and Figure 1. Discoloration generally decreased as age of wound decreased in Series 1 trees where the oldest wounds of 12 mo were initiated during the dormant season. Discoloration generally increased, however, as age of wound decreased in Series 2 trees where the oldest wounds were initiated during the growing season. Thus, discoloration associated with wounds initiated during the growing season was significantly less than that associated with wounds initiated during the dormant season, regardless of wound age. Significantly less discoloration, however, was associated with August than with May wounds of both series. This within growing-season variation could be related to the additional age of 3 mo of the May wounds.

Clones differed in their capacity to compartmentalize as reflected by the amount of discoloration associated with wounds. Mean discoloration associated with wounds initiated in all seasons in clones WI 1, Alt 1, and St 74 was significantly less than that in clones St 109, St 75, and St 92 (Table 2). Ethylene production by increment cores collected in February correlated best with the ranking for mean clonal discoloration, i.e., $r = 0.83$ for clonal discoloration and ethylene production during the first day of incubation (et_1). This correlation did not hold for the other seasons. In an analysis of covariance, an $R^2 = 0.81$ was achieved with the model log discoloration = clone, tree within clone, log et_1, season, and clone × season interaction. All of these factors except

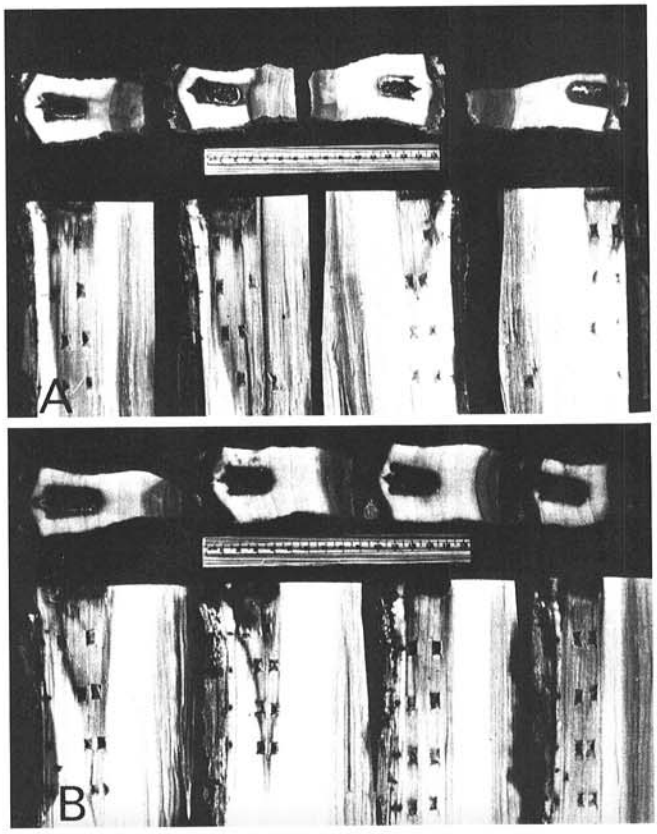

Fig. 1. Cross (upper) and longitudinal (lower) sections through wounds 12, 9, 6, and 3 mo old (left to right) in two ramets of *Populus deltoides* clone Wickliffe 1. **A,** The oldest wound in this ramet was initiated at the beginning of the dormant season (November). More discoloration of the longitudinal sections was associated with wounds 12 and 9 mo old than 6 and 3 mo old, giving the impression that increased discoloration was related to increased wound age. But in **B,** the oldest wound in this ramet was initiated at the beginning of the growing season (May). Older wounds, i.e., 12 and 9 mo, initiated during May and August had less associated discoloration than younger wounds initiated during the dormant season. Season of wounding therefore influenced the amount of associated discoloration more than wound age.

TABLE 1. Effect of wound age and season of wounding on wound-associated discoloration of eastern cottonwood

Month of wound	Age of wound (mo)	Mean Discoloration[z] (%)
Series 1 (11/84 harvest)		
11/83	12	93.0 a
2/84	9	94.5 a
5/84	6	73.4 b
8/84	3	43.7 d
Series 2 (5/85 harvest)		
5/84	12	77.5 b
8/84	9	52.3 c
11/84	6	92.0 a
2/85	3	95.9 a

[z] Each value is the geometric mean of 18 observations. Means followed by the same letter are not significantly ($P = 0.05$) different as determined by multiple comparisons of least square means.

TABLE 2. Wound-associated discoloration and ethylene production by cores of outer xylem 1 and 2 days after their excision from clones of eastern cottonwood in four seasons

Clone	Mean discoloration[x] (%)	Ethylene production (nl g^{-1} dry wt hr^{-1}) November Day 1	November Day 2	February Day 1	February Day 2	May Day 1	May Day 2	August Day 1	August Day 2
Wi 1	63.6 a	8.7 ab[y]	21.9 ab	4.7 bc	18.0 bc	19.4 a	17.4 ab	11.6 ab	18.6 b
Alt 1	68.0 a	5.3 c	18.4 ab	4.0 cd	24.3 ab	10.6 b	14.2 b	8.9 abc	16.7 b
St 74	70.1 a	7.2 bc	24.6 a	2.2 d	24.5 ab	12.4 ab	19.8 ab	8.4 bc	30.0 a
St 109	80.6 b	9.5 ab	17.0 b	7.0 abc	8.3 d	11.4 b	16.3 ab	12.5 a	16.2 b
St 75	82.4 b	8.0 bc	17.9 b	8.8 a	32.5 a	12.5 ab	25.0 a	6.5 c	18.4 b
St 92	88.8 b	12.6 a	17.6 b	8.3 ab	15.1 c	13.8 ab	8.7 c	10.8 ab	13.8 b
Seasonal means[z]									
	Day 1	8.3 s		5.3 r		13.1 u		9.6 t	
	Day 2		19.4 w		18.8 w		16.1 v		18.4 vw

[x] Each value is the geometric mean of 24 observations. Means followed by the same letter are not significantly ($P = 0.05$) different as determined by multiple comparisons of least square means.

[y] Each value is the geometric mean of six observations. Means in the same column followed by the same letter are not significantly ($P = 0.05$) different as determined by multiple comparisons of least square means.

[z] Each value is the geometric mean of 36 observations. Means in the same row followed by the same letter are not significantly ($P = 0.05$) different as determined by multiple comparisons of least square means.

TABLE 3. Seasonal initiation and closure of wounds in stems of eastern cottonwood

Month of wound	Closure[y] at month of observation Feb	May	Aug	Nov	Feb	May
Series 1						
Nov	1.0 a[z]	1.2 a	3.9 c	4.0 c		
Feb		1.4 a	4.0 c	4.0 c		
May			3.9 c	3.9 c		
Aug				1.5 a		
Series 2						
May			3.9 c	3.9 c	3.9 c	4.0 c
Aug				1.5 a	1.5 a	3.6 c
Nov					1.0 a	2.7 b
Feb						3.5 c

[y] Wound closure was rated on a scale of 1–4: 1 = wound completely open (1.27 cm diameter), 2 = wound one-third closed, 3 = wound two-thirds closed, 4 = wound completely closed.

[z] Each value is the mean of 18 observations. Means followed by the same letter are not significantly ($P = 0.05$) different as determined by multiple comparisons of least square means.

TABLE 4. Wound closure by clones of eastern cottonwood 3 and 6 mo after wounding

Clone	Wound closure[y] 3 mo	6 mo
St 92	1.90 a[z]	2.50 a
St 109	2.10 ab	2.70 b
Alt 1	2.10 b	2.80 b
St 74	2.25 bc	2.85 b
Wi 1	2.30 c	3.20 c
St 75	2.60 d	3.20 c

[y] Wound closure was rated on a scale of 1–4: 1 = wound completely open (1.27 cm diameter), 2 = wound one-third closed, 3 = wound two-thirds closed, 4 = wound completely closed.

[z] Each value is the mean of 24 observations 3 mo and 18 observations 6 mo after wounding. Means followed by the same letter are not significantly ($P = 0.05$) different as determined by multiple comparisons of least square means.

tree within clone were highly significant ($P = 0.0001$). When clone was deleted from the model, however, log et_1 was no longer significant in predicting log discoloration but season remained highly significant. Thus clone and season of wounding were the most important factors in this model for determining discoloration.

Seasonal means across clones of first-day ethylene production (et_1) differed significantly from each other. Highest amounts were produced in May and lowest amounts in February. Second day ethylene production (et_2) was higher than et_1, but seasonal means of et_2 across clones did not differ widely, although May values were significantly lower than those of November and February.

Little or no ethylene was detected in wounds at the time of wounding (level of detection about 0.05 ppm). Measurable amounts of methane, however, were detected frequently in gases evacuated from wounds at the time of wounding. Methane is a product of anaerobic methanogenic bacteria that inhabit the interior wetwood of poplars and other tree species (24). Almost all of the methane diffused from increment cores within the first day of incubation. The amount of methane that emanated from cores was sometimes considerable (i.e., as high as 42.8 μl g^{-1} dry wt for a ramet of clone 92 in August), but it did not correlate well with subsequent compartmentalization or closure. There was a tendency, however, toward negative correlation between methane and these host responses. Methane emanations from excised cores were significantly lower in May than in the other seasons.

Wounds largely closed during the 3-mo period from May to August (Table 3). There was a tendency for February wounds to close more rapidly than November wounds. This could reflect the additional exposure of November wounds to debilitating factors, including microorganisms, during the dormant season. The probable reason for significantly slower closure of wounds made in February and observed in May in Series 1 compared to Series 2 (i.e., 1.4 vs. 3.5) is that observations for Series 2 were made in late rather than mid May.

Clones differed significantly when their mean wound closures over all seasons for 3 and 6 mo were compared (Table 4). Closure was significantly more advanced 6 mo after wounding in clones St 75 and WI 1 than in clones St 74, Alt 1, St 109, and St 92. Closure of wounds in clone St 92 was significantly slower than that for all other clones tested. These trends were similar for the pooled observations made 3 mo after wounding. There was a tendency for more rapid wound closure in those clones with higher et_2 values (Table 2) in May.

Some patterns of colonization became evident even though no attempt was made to identify all fungi isolated from wounds. The influence of season of wounding and wound age on the frequency of isolation of some fungi is shown on Table 5. A highly significant ($P = <0.005$) "G-test" of independence (20) was calculated for the effect of season of wounding on the proportion of wounds colonized by *Fusarium solani* or decay fungi. Thus, *F. solani* was isolated significantly more frequently from wounds initiated during the growing season (May, August) than during the dormant season (November, February), regardless of wound age. Decay fungi, on the other hand, were isolated significantly more

frequently from wounds initiated during the dormant season than during the growing season, regardless of wound age. Age of wound was related less significantly ($P = <0.05$) than season of wound to the proportion of these fungi isolated from wounds. Their incidence tended to decrease as wound age increased.

Among the decay fungi isolated, with decreasing frequency, were *Bjerkandera adusta* (*Polyporus adustus*), *Phlebia* sp., *Phanerochaete flavido-alba*, *Chondrostereum purpureum* (*Stereum purpureum*), *Coriolus versicolor*, *Stereum gausapatum*, *Stereum complicatum*, *Coprinus micaceus*, *Oxyporus latemarginatus*, and *Sistotrema brinkmannii*.

DISCUSSION

The experimental design permitted the study of dynamic host responses to wounds initiated on the same clones during each season but differing from 3 to 12 mo in wound age. By offsetting the two wounding series by 6 mo, it was possible to isolate the effect of wound age from season of wounding. During this experimental period, season of wounding influenced the amount of wound-associated discoloration far more than wound age. Thus, wounds initiated during the growing season (May-August) compartmentalized significantly better than wounds initiated during the dormant season (November-February) regardless of wound age. This and other aspects of the study emphasize the importance of taking season into account in studies dealing with pathogen-perennial host interactions.

Significantly less discoloration was associated with August wounds than with May wounds. This within growing-season variation could be related to the additional age of 3 mo for the August wounds in both wounding series. But this does not explain why 6-mo-old wounds initiated in May (Series 1) had significantly more associated discoloration than 9-mo-old wounds initiated in August (Series 2). This implies that August wounds compartmentalize better than May wounds even though May wounds were largely closed by August, whereas August wounds remained opened until the following growing season. Rapid wound closure, therefore, was not related to decreased microbial colonization. Our mycological study was too limited to suggest that the suite of fungi that invaded May wounds was more aggressive than that which invaded the August wounds. Indeed, decay fungi were isolated less frequently from May than from August wounds.

Season of wounding seemed more influential than age of wound with regard to fungal colonization of wounds. Thus, *F. solani* frequently colonized wounds initiated during the growing season and a variety of decay fungi preferentially colonized wounds initiated during the dormant season. These fungi were isolated less frequently with increasing wound age. These results, therefore, do not support the concept of a succession of microorganisms in wounded xylem leading from non-Hymenomycetes to Hymenomycetes with increasing wound age (16). Rather, it appeared that wounds were colonized by inoculum available at the time of wounding, including that of Hymenomycetes and that the frequency of these fungi decreased with increasing time. A similar decrease in the frequency of the decay fungus *Heterobasidion annosum* with increasing wound age was observed earlier (9,12).

TABLE 5. Frequency of some fungi in seasonal wounds of different ages in eastern cottonwood

Season	Months of wounding	Age of wounds (mo)	Frequency of fungi[x]	
			Decay fungi (%)	*Fusarium solani* (%)
Dormant	Nov, Feb	3,6	50 a	39 c
		9,12	25 a	8 d
Growing	May, Aug	3,6	17 b	83 e
		9,12	11 b	67 e

[x] Each value is the percentage of 36 wounds that contained the fungi designated. Means in the same column followed by the same letter are not significantly ($P = 0.05$) different as determined by the G-test of independence (20).

Seasonal differences in wound colonization by fungi were reported earlier (9,10). As perhaps expected, these differences can vary with fungal species and host location. *B. adusta* was mentioned prominently as an invader of late-spring frost wounds in oak in Poland (1), whereas it was isolated least in May wounds and most in November wounds in the present study. This fungus also colonized increment-borer wounds in eastern cottonwood in Mississippi where, with other decay fungi, it was isolated from 12.5% of fall wounds but not from spring wounds 2 yr after wounding (23). Shigo (17), on the other hand, reported that Hymenomycetes were isolated within 7–10 wk after initiation of summer wounds but not until 5.5 mo after initiation of winter wounds in red maple in Maine. The availability of inoculum in a given season and the capacity of a given fungus to compete with others for wounded host tissue could be major factors in wound colonization.

Few studies have compared the growth of a specific Hymenomycete after inoculation in different seasons. In one such study (12), sapwood of loblolly pine was inoculated with *H. annosum* during the dormant and growing seasons. The longitudinal penetration 3 mo after inoculation was 11.3 and 0.9 cm in the dormant and growing seasons, respectively. This and other evidence was presented for the association of sapwood resistance to its enhanced physiological activitity during the growing season.

The seasonal course of et_1 faithfully mirrored that expected for physiological activity of sapwood. That is, ethylene production by cores excised at different seasons but incubated at the same temperature for 1 day increased significantly through February, November, August, and May ($P = 0.05$). Excised cores collected in February produced significantly less ethylene in good than in poor compartmentalizing clones. This may not seem meaningful biologically until this basal rate of ethylene production in February is compared to its maximal rate in May. Thus, ratios of basal (February) to maximal (May) rates of first-day ethylene production ranged from 2.7 to 5.6 for better compartmentalizing clones and from 1.4 to 1.7 for the poorer compartmentalizing clones. Using similar rationale, i.e., that better compartmentalizers react substantially greater above basal levels than poorer compartmentalizers, a ratio of second to first day ethylene production during February also may be of interest. In the present study, day 2:day 1 ratios ranged from 3.8 to 11.1 and from 1.2 to 3.7 for the good and poorer compartmentalizers, respectively.

These results suggest that it may be possible to predict a clone's capacity to compartmentalize on the basis of ethylene production by excised cores of viable sapwood. Additional testing with a larger sample size, however, will be necessary to confirm or refute this hypothesis. It seems that a ratio of ethylene determinations may have better predictive value than a single determination because relationships can be drawn from the former, whereas the latter only represents a point observation that could be affected greatly by environmental conditions (8). Excised cores were not treated with test substances in the present study. Ethylene ratios generated from replicated isogenic cores that are treated or not treated with metabolites of wound-colonizing fungi or perhaps precursors of ethylene may enhance the accuracy of this putative screening system.

A possible role of ethylene in the compartmentalization process could be in the mediation of the highly tylosed, phenol-enriched zone that surrounds decay columns and wound-associated discoloration in this species. Direct evidence for this hypothesis, however, has not yet been presented.

Rapid wound closure was not predictive of good compartmentalization, e.g., closure was most rapid in a clone (St 75) that compartmentalized poorly. This suggests, in agreement with Garrett et al. (2), that these two events are under separate genetic control. Wound closure was related to radial growth of trees in some studies (6,7) but not in others (2). Using radial growth rate of clones during ages 5–10 yr in the same plantation (22) and wound closure at 3 mo (Table 4), an r of 0.85 could be calculated.

Ethylene stimulated wound healing and callus production in other higher plants (4,21). There is some evidence in the present

study to indicate that wound closure was related to ethylene production by sapwood excised at the beginning of the growing season. This relationship may have been stronger had the cambial layer not been removed before core incubation. It is possible, therefore, that ethylene may play a role in the mediation of both compartmentalization and wound closure.

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