論 文

Ethylene Production by Bark Segments Taken from Areas around Mechanical Wounds on Stems of *Cryptomeria japonica* D. Don.

Fukuju YAMAMOTO*
Kazutami KASAI**
Hayato HASHIZUME*

スギ樹幹の傷害部周囲におけるエチレンの生成

山 本 福 壽*
笠 井 和 民**
橋 詰 隼 人*

Summary

The effects of wound stimuli on acceleration of ethylene production by bark segments of *Cryptomeria japonica* extended to at least 5 cm away from the edges of 2×2 cm square hammered or bark-peeled wounds along the upper and the lower directions at 12 hours after wounding. Along the lateral direction, however, accelerated ethylene emanation was detected as far as 2 cm away from the wound edges. Ethylene production was slightly higher in the hammered wounds than in the bark-peeled wounds. The enhanced ethylene production continued for at least 7 days. The treatment of ACC, an immediate precursor of ethylene, greatly accelerated ethylene release from bark discs, whereas AVG, an inhibitor of ACC synthesis, significantly suppressed ethylene synthesis in the bark discs. The range of wound effects on stress ethylene production around the wounded area is discussed.

I INTRODUCTION

It has been postulated that stress ethylene plays an important role in modification of growth and morphology of stems of woody species[1]. Ethylene production by stems increases as a result of various stresses including flooding[2,3,4,5], gravity[5,6,7], mechanical bending[8,9,10], injection of chemicals[11], insect attack[12], and wounding[13,14,15]. Vast emanation of ethylene by mechanically-

* Laboratory of Silviculture, Department of Forestry Science, Faculty of Agriculture, Tottori University
** Koshii Mokuzai Co. Ltd.

wounded phloem and xylem tissues *in vitro* and *in vivo* has been well documented for coniferous species[13,14,15]. Such enhancement of ethylene production is considered to be correlated with the formation of traumatic phloem resin-canals[13,15], oleoresin production[16], and synthesis of phenolics related to heartwood formation[17,18]. However, the direct regulatory roles of ethylene in wound healing and morphological changes associated with the defense against invading microorganisms are still obscure. In the present study, the range of wound effects on stress ethylene production around the wounded area was investigated.

II MATERIALS AND METHODS

1. Plant materials

Eleven 12-year-old *Cryptomeria japonica* D. Don trees (Boka-sugi clone) growing in the Hiruzen forest of the Tottori University forest were selected for uniformity of heights and diameters of breast-height. Average values of tree height and stem diameter at the beginning of the experiment were 850 cm and 10.5 cm, respectively.

2. Experiment 1

On June 16, 1988, six bark-punched wounds 2.5 cm in diameter were made with a punch at intervals of at least 50 cm on each stem of the two trees. Then, three different bark discs 2.5 cm in diameter were sampled for determination of ethylene production using the punch at three different portions around adjacent to the wound. Six-replicated sampling was carried out in each tree at 12 and 24 hours after wounding. The reverse side of the wounded portions of the stems was used for taking bark discs for control (Fig. 1).

3. Experiment 2

On June 21, six bark-peeled wounds of 2×2 cm square were made on the stem of one of the two trees with a razor blade. In another tree, six hammered wounds of 2×2 cm square were also made by hitting 20 times with a 370 g iron hammer the end of a 20-cm long and 2-cm in diameter scantling attached to the stem. After 12 hours, six-repeated, five different pieces of 1×2 cm bark segments were taken along each of the upper, the lower, and the lateral direction at 0-1, 1-2, 2-3, 3-4, and 4-5 cm from the edges of the peeled wounds (Fig. 1). The same sampling was also made around the hammered wounds. Six-repeated, one bark segment of the same size was also taken from the hammered spot. The reverse side of the wounded portions of the stems was used for taking control segments.

To determine the duration of ethylene production, four bark-peeled wounds of 2×2 cm square also made on each stem of 6 trees with a razor blade on September 15. One 2×1 cm bark

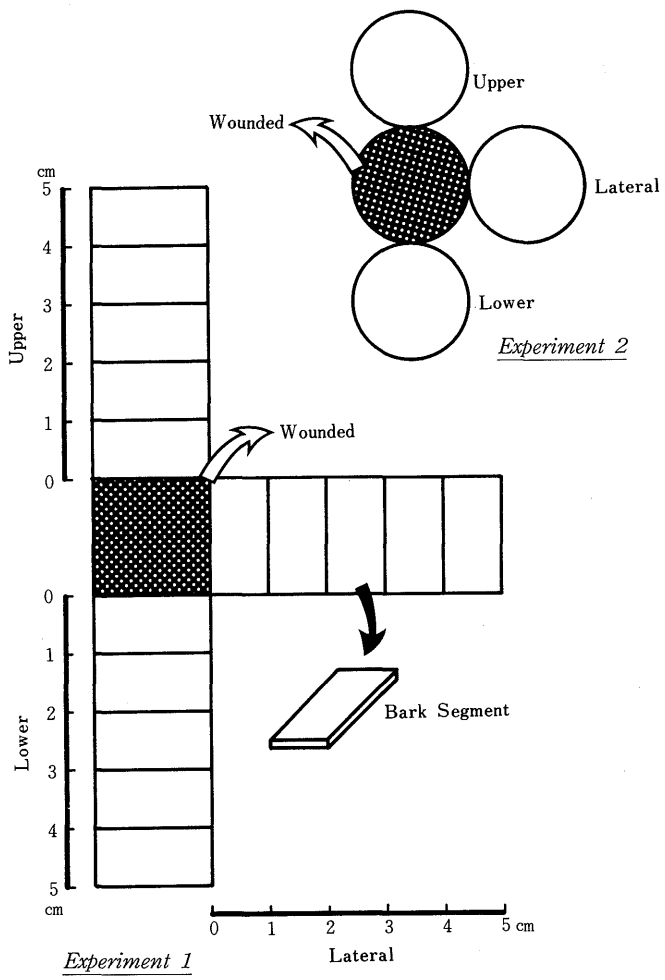

Fig. 1 Diagrams illustrating appearance and locations of wounds and bark discs as well as segments taken for ethylene meanurement in experiment 1 and experiment 2.

segment was taken at 1 to 2 cm below the edge of the bark-peeled wound of each of the 6 trees at 1, 3, 5, and 7 days after wounding. Bark segments for controls were taken at the reverse side of the wounded portions of the stems.

4. Experiment 3

On July 1, a 200-cm long and about 10-cm in diameter stem piece was taken into the laboratory at 2 hours after cutting. Bark discs, 25-mm in diameter and about 1.1mm thick, were immediately prepared by punching the bark of the stem piece with the punch. Six discs were placed in a 100-ml beaker and dipped in each 20-ml test solution of methionine (MET), 1-aminocyclopropane-1-carboxylic acid (ACC), and aminoethoxyvinylglycine (AVG) as indicated in Table 2 and 3 for 10 minutes at 5°C. Then, each disc was dried with a piece of Wako №2

filter paper and transferred into a 15-ml vial for ethylene determination.

5. Ethylene determination

Ethylene was determined using a modification of the method proposed by Yamamoto et al.[3] Each bark disc or segment was placed in a 15-ml vial containing 0.5-ml of saturated ammonium sulfate and sealed with a rubber stopper. The samples were incubated in a water bath at 31°C for 20h. Aliquots (1 ml) of head space gas were sampled at intervals and analyzed for ethylene using a Hitachi 263-50 gas chromatograph with a flame ionization detector (FID) and a spiral glass column (0.3×200 cm) packed with 60/80 activated alumina. Chromatograph conditions were as follows: column, injector, and detector temperatures, 70°C, 80°C, and 100°C, respectively; carrier gas (N_2), 35 ml/min. Measurements of ethylene were computed from standard curves calibrated from known concentrations of ethylene-nitrogen mixtures (47.9 ppm). The amount of ethylene released by bark discs or segments (nmol/gDW) was calculated as:

$$(Vt+Vg-Vs)\times Ce/DW$$

where Vt=volume of vial containing bark segment (ml)

Vg=volume of sample gas from vial (ml)

Vs=volume of bark disc or segment (ml)

Ce=ethylene concentration in the vial (nmol/ml)

DW=dry weight of the bark disc or segment (g)

III RESULTS

Wounding greatly stimulated ethylene release from bark discs taken from around the wounded portions in experiment 1. In Table 1, a fairly high amount of ethylene production was obtained at 12 hours after wounding in all of the discs around the wounds. The high ethylene content was detected in the upper and the lower portions of the wounds rather than in the lateral portions. The ethylene emanation, however, significantly decreased at 24 hours.

Table 1 Wound ethylene production by 2.5-cm diameter bark discs taken from the upper, the lower, and the lateral portions adjacent to the bark-punched wounds. Bark discs for controls were taken at the opposite side of the wounded portions of the stems. The discs were taken at 12 and 24 hours after wounding. Each value is the mean ± SE of 6 replications. (Experiment 1).

The time elapsed	Ethylene (nmol/gDW)			
Hours	Cont.	Upper	Lower	Lateral
12	17.4±5.1	338.9±32.2**	321.2±45.9**	198.4±29.5**
24	17.4±5.2	119.7±14.3**	108.9±20.0**	27.6±4.1ns

**. significantly different from controls at the 1 % level ; ns. not significant.

In experiment 2, ethylene emanation from bark segments was fairly remarkable at the upper and the lower portions of the wounds in comparison with the lateral portions (Fig. 2). The ethylene production was slightly higher in the hammered wounds than in the bark-peeled wounds in all of the three portions. At 12 hours after hammering, the highest ethylene production was detected at 1 to 3 cm away from the wound edges along the upper and the lower directions. However, ethylene emanation by the hammered spots was very poor. In the bark-peeled wounds, high ethylene production was measured in all of the segments taken at 1 to 5 cm away from the edges along the upper and the lower directions. The wound effect on ethylene production did not extend beyond 2 cm away from the edges along the lateral direction.

Table 2 indicates the duration of wound ethylene production at 1 to 2 cm below the wounded portions. The high level of ethylene was detected for at least 7 days.

Table 2 Wound ethylene production by 2.0 × 1.0 cm bark segments taken at 1 to 2 cm below the edge of the bark-peeled wounds. Bark segments for controls were taken at the reverse side of the wounded portions of the stems. The samples were taken at 1, 3, 5, and 7 days after wounding. Each value is the mean ± SE of 6 replications. (Experiment 2).

The time elapsed	Ethylene (nmol/gDW)	
Days	Cont.	Lower
1	48.8± 6.9	111.3±34.0ns
3	63.7± 8.7	166.6±41.8*
5	62.3±11.4	100.0±22.6ns
7	83.8±13.0	188.4±30.3*

*, significantly different from controls at the 5 % level ; ns, not significant.

Two precursors of ethylene greatly affected ethylene release from bark discs (Table 3). The treatment of 0.1 and 1.0 mmol ACC significantly accelerated ethylene production, whereas 1.0 mmol methionine produced only slight stimulation.

Table 4 shows the effects of different concentratioss of AVG on ethylene emanation. Even a low concentration of AVG (0.001 nmol) significantly suppressed ethylene synthesis in the bark discs.

IV DISCUSSION

In the present study, ethylene emanation by bark segments adjacent to the wounds was much greater at 12 hours than at 24 hours. These results are consistent with Yamanaka's observations[15] that ethylene production in vitro by bark segments of *Crypomeria* reached maximum rates at 12 hours after sampling.

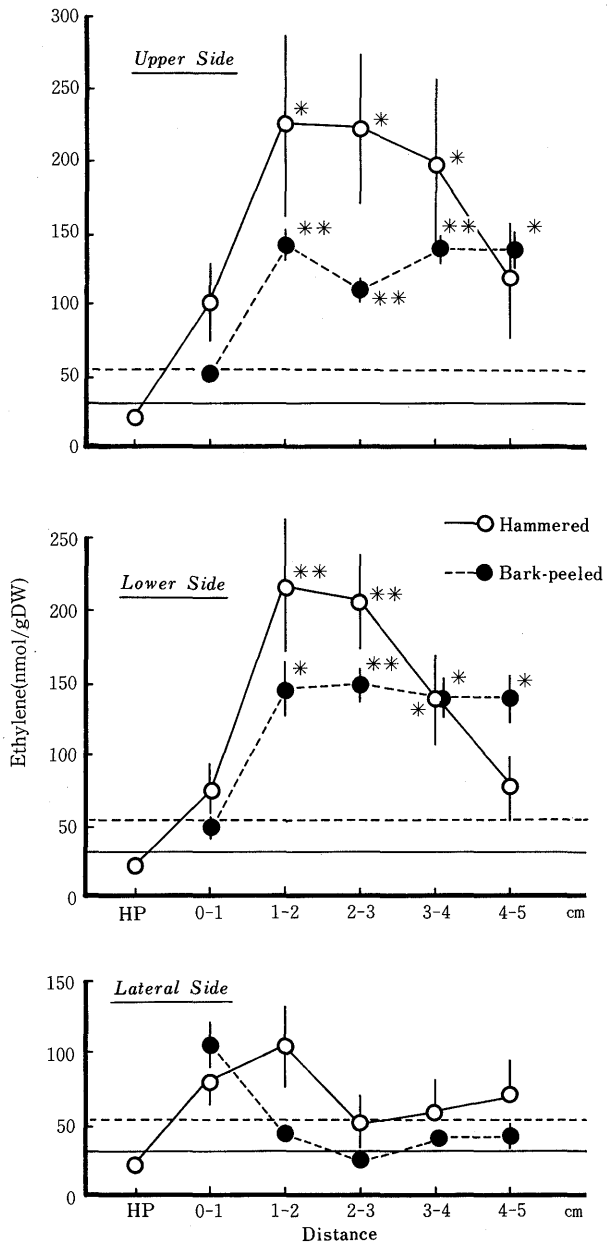

Fig. 2 Wound ethylene production measured for 5 different 1 × 2 cm bark segments taken from each of the upper, the lower, and the lateral portion on the wounds at 1-cm intervals from the wound edges. A bark segment of the same size was also taken from the hammered portion. All of the segments were taken at 12 hours after wounding. Bars indicate SE of the mean (n=6). Details : dotted line = control value for bark-pealed wounds (54.7 ± 1.6 nmol/gDW) : solid line = control for the hammered wounds (31.9 ± 4.2 nmol/gDW) : HP = hammered portion. (Experiment 2).

*, significantly different from controls at the 5% level; **, at the 1% level; ns, not signficant.

Table 3 Effects of methionine (MET) and 1-aminocyclopropane-1-carboxytic acid (ACC) on ethylene production by bark segments. Each value is the mean ± SE of 6 replications. (Experiment 3)

Concentrations of C_2H_4 Precursors	Ethylene (nmol/gDW)
MET (nmol/l)	
0 (Cont.)	10.8±1.3
0.01	12.9±2.9ns
0.1	21.5±7.1ns
1.	21.1±3.2*
ACC (nmol/l)	
0.01	29.3± 8.5ns
0.1	36.9± 6.1**
1.	120.6±18.0**

*. significantly different from controls (0 nmol) at the 5 % level ; **. at the 1 % level ; ns, not significant.

Table 4 Effect of aminoethoxyvinylglycine (AVG) on ethylene production by bark segments. Each value is the mean ± SE of 6 replications. (Experiment 3)

Concentration of AVG (nmol/l)	Ethylene (nmol/gDW)
0 (Cont.)	4.2±0.4
0.001	2.8±0.3*
0.01	2.0±0.1**
0.1	0.7±0.1**

*, significantly different from controls (0 nmol) at the 5 % level ; **, at the 1 % level.

Yamanaka[13] also investigated ethylene production *in vivo* by wounded phloem tissue of *Chamaecyparis obtusa* as well as *in vitro* by phloem plus cambium segments. To interpret roles of ethylene on wound healing, as Yamanaka[15] mentioned, the measurement of ethylene production *in vivo* may be more practical than *in vitro*. In our present data, wound effects on ethylene production by bark segments extended to at least 5 cm away from wound edges along the upper and the lower directions at 12 hours after wounding. Along the lateral direction, however, accelerated ethylene emanation was detected at 2 cm away at most. Further, such enhanced ethylene production continued for at least one week after wounding. These data imply that the wound stimuli on ethylene production were zonally propagated to both upper and lower directions from the wounded area through the bark tissue. For investigations of the physiological mechanisms of wound healing and morphological and functional changes associated with the defense against invading microorganisms, it is suggested that tracing the extent, the duration, and the intensity of wound effects on ethylene production around the wounded area

may be more important than measuring sequentialethylene production by wounded spots or by isolated bark segments.

The range of ethylene production in the present data may be associated with the fusiform expanse of traumatic phloem resin-canals along the stem axis induced by insect attack or the treatment of toxic chemicals in *Cryptomeria*[19]. The formation of xylem resin-canals was also induced by endogenous and exogenous ethylene in Pines[3,4]. However, high concentrations of exogenous ethylene significantly suppressed or delayed the formation of traumatic phloem resin-canals in wounded stems of *Chamaecyparis obtusa,* whereas this was accelerated by the removal of ethylene[15]. These phenomena suggest that optimal concentrations of internal ethylene for the formation of phloem resin-canals might be fairly low in *Chamaecyparis,* if ethylene involves formation of traumatic resin-canals generally in coniferous species.

Rapid ethylene production was detected in ACC-treated bark segments of *Cryptomeria,* whereas AVG significantly suppressed ethylene synthesis. These observations are consistent with the finding that wounding induces synthesis of ACC synthase and causes accumulation of ACC and an increase in ethylene production[20].

Further investigations on the physiological roles of wound ethylene in morphological, anatomical, and functional modifications of injured stems of coniferous species are needed to interpret wound-healing mechanisms.

REFERENCES

1) Savidge, R. A. : Auxin and ethylene regulation of diameter growth in trees. Tree Physiology, 4: 401-414 (1988).

2) Tang, Z. C., and T. T. Kozlowski: Ethylene production and morphological adaptation of woody plants to flooding. Can. J. Bot. 62: 1659-1664 (1984).

3) Yamamoto, F., T. T. Kozlowski, and K. E. Wolter: Effect of flooding on growth, stem anatomy, and ethylene production of *Pinus halepensis* seedlings. Can. J. For. Res. 17: 69-79 (1987).

4) Yamamoto, F. and T. T. Kozlowski: Effects of flooding, tilting of stems, and enthrel application on growth, stem anatomy and ethylene production of *Pinus densiflora* seedlings. J. Exp. Bot. 38 (187): 293-310 (1987).

5) Yamamoto, F, and T. T. Kozlowski: Effects of flooding, tilting of stems, and ethrel application on growth, stem anatomy and ethylene prtduction of *Acer platanoides* seedlings. Scand. J. For. Res. 2: 141-156 (1987).

6) Robitaille, H. A.: Stress ethylene production in apple shoots. J. Amer. Soc. Hor. Sci. 100 (5): 524-527 (1975).

7) Nelson, N. D. and W. E. Hillis.: Ethylene and tension wood formation in *Eucalyptus*

gomphocephala. Wood. Sci. Technol. 1 2 : 309-315 (1978).

8) Brown, K. M. and A. C. Leopold: Ethylene and regulation of growth in pine. Can. J. For. Res. 3: 143-145 (1973).

9) Leopold, A. C., K. M. Brown, and F. H. Emerson: Ethylene in the wood of stressed trees. HortScience. 7 (2): 175 (1972).

10) Robitaille, H. A. and A. C. Leopold: Ethylene and the regulation of apple stem growth under stress. 32: 301-304 (1974).

11) Wolter, K. E.: Ethylene-potential alternative to bipiridilium herbicides for inducing light wood in red pine. Proc. Annu. Lightwood Res. Conf. 90-99 (1977).

12) Shain, L. and W. E. Hillis.: Ethylene production in *Pinus radiata* in response to sirex-amylostereum attack. Phytopathol. 62: 1407-1409 (1972).

13) Yamanaka, K.: Ethylene production in *Chamaecyparis obtusa* phloem and xylem tissues in response to wounding. Mokuzai Gakkaishi. 31 (9): 703-710 (1985).

14) Yamanaka, K.: Wound ethylene production by phloem and cambium tissues in conifers. Mokuzai Gakkaishi. 32 (2): 136-139 (1986).

15) Yamanaka, K.: Studies on morphological and physiological responses of stem tissues to wounding in conifers. His phD thesis (Kyoto University). (1986)

16) Wolter, K. E. and D. F. Zinkel: Observation on the physiological mechanisms and chemical constituents of induced oleoresin synthesis in *Pinus radiata.* Can. J. For. Res. 14: 452-458 (1984).

17) Shain, L. and W. E. Hillis.: Ethylene production in xylem of *Pinus radiata* in relation to heartwood formation. Can. J. Bot. 51: 1331-1335 (1973).

18) Phelps, J. E., N. D. Nelson, W. J. Rietveld, and E. A. McGinnes, Jr.: Rates of ethylene production by parenchyma cells in black walnut sapwood. Wood and Fiber Sci. 15: 23-27 (1983).

19) Yamanaka, K.: Normal and traumatic resin-canals in the secondary phloem of conifers. Mokuzai Gakkaishi. 30 (5): 347-353 (1984).

20) Yu, Y. B, and S. F. Yang.: Biosynthesis of wound ethylene. Plant. Physiol. 66: 281-285 (1980).