Plant Physiol. (1978) 62, 710–712

Rapidly Induced Wound Ethylene from Excised Segments of Etiolated *Pisum sativum* L., cv. Alaska

III. INDUCTION AND TRANSMISSION OF THE RESPONSE[1]

Received for publication February 23, 1978 and in revised form July 6, 1978

MIKAL E. SALTVEIT, JR.,[2] AND DAVID R. DILLEY
Department of Horticulture, Michigan State University, East Lansing, Michigan 48824

ABSTRACT

Increased ethylene synthesis was rapidly induced throughout the apical meristematic region of etiolated seedlings of *Pisum sativum* L., cv. Alaska by cuts made 1 centimeter from the apical hook. The wound signal was transmitted at about 2 millimeters per minute. Accumulation of substance(s) at the cut surfaces of excised sections, as the result of interrupted translocation, did not initiate or significantly contribute to wound-induced ethylene synthesis, nor was the cut surface the site of enhanced ethylene synthesis. Cutting subapical sections into shorter pieces showed that cells less than 2 millimeters from a cut surface produced about 30% less ethylene than cells greater than 2 millimeters from a cut surface.

Previous studies by Saltveit and Dilley (15, 16) showed a rapidly induced, transitory increase in ethylene synthesis in tissue excised from the actively growing regions of seven species of etiolated mono- and dicotyledonous seedlings. The kinetics of wound-induced ethylene synthesis varied with the region of the plant from which the tissue segment was excised. The kinetic behavior was characterized for 9-mm subapical stem segments excised 9 mm from the top of the apical hook, from etiolated 7-day-old seedlings of *Pisum sativum* L., cv. Alaska. Wound-induced ethylene production at 25 C started to increase linearly after a lag period of 26 min from 2.7 nl g^{-1} hr^{-1} to the first maximum of 11.3 nl g^{-1} hr^{-1} at 56 min. The rate of production then decreased to a minimum at 90 min, increased to a lower second maximum at 131 min, and then declined over a period of about 100 min to about 4.0 nl g^{-1} hr^{-1}.

If plants are subjected to severe stress ethylene biosynthesis is diminished or completely abolished while moderate stress is stimulatory (1, 14, 17). Ethylene synthesis was stimulated by cutting apples (13), bananas (12), tomatoes (10), sweet potato roots (4), bean petioles (6), and etiolated rice coleoptiles (5) into pieces. However, mincing or homogenizing the tissue reduced or stopped ethylene production (4, 10, 13). Jackson and Osborne (6) reported that cutting 2-cm petiole segments of *Phaseolus vulgaris* L., cv. Canadian Wonder into smaller pieces stimulated ethylene production in proportion to the number of cut surfaces. Increasing the cut surface area of sweet potato roots (*Ipomoea batatas* L., cv. Norin 1) stimulated ethylene production in proportion to the logarithm of the area (4). In contrast, cutting etiolated rice (*Oryza sativa* L., cv. Aichi-Asahi) coleoptiles into smaller segments stimulated ethylene production, but the increase was not proportional to the number of cut surfaces (5). Laties (8) reported that increased respiration occurred initially throughout a potato tuber slice, and the duration of the rise in any part of the slice was dependent on the distance from the cut surface.

Ethylene and ethane production data from sugar beet (*Beta vulgaris* L.) leaf discs frozen by a 3-mm-diameter stainless steel rod kept at the temperature of liquid N_2 (3) can be used to show that cells up to about 0.5 mm from the edge of the frozen spot were stimulated to make more ethylene. This compares to an activated zone of less than 3 mm from the cut surface of bean petiole sections (6).

This study characterizes the kinetics of the induction of wound-induced ethylene synthesis by etiolated "Alaska" pea stem segments.

MATERIALS AND METHODS

Plant Material. Seven-day-old etiolated seedlings of "Alaska" pea were grown and prepared as previously described (15, 16). Apical stem segments included the apical meristem, plumule, apical hook, and a portion of the stem. They were cut to lengths of 130 mm or 27 mm from the top of the apical hook. All kinetic studies of wound-induced ethylene synthesis were done with 9-mm subapical stem sections excised 9 mm from the top of the apical hook. Ethylene was analyzed as previously described (15, 16).

Induction and Transmission of Wound Signal. Transmission of the wound signal was investigated using 9-mm subapical sections excised from 130-mm or 27-mm apical stem segments freshly harvested or held in a humid, ethylene-free atmosphere for 15 min. Subapical sections were excised at time zero or 15 min, respectively, and the kinetics of wound ethylene evolution studied for 90 min.

Development of polarity in wound-induced ethylene synthesis was studied with subapical sections left whole or cut into apical and basal halves at 0, 15, 30, 60, or 90 min after excision. The halved tissue was enclosed in 25-ml Erlenmeyer flasks on moist filter paper and CO_2 was absorbed with KOH. Gas samples were analyzed for ethylene 30 min after time zero, and at 60 min after all other times.

The possible induction of wound ethylene synthesis by accumulation of translocatable substance at the cut surface was studied by removing 3 mm from the apical or basal end of a 16-mm subapical section. The 16-mm section was excised 6 mm from the top of the apical hook, so that after removal of the apical and basal 3-mm sections of stem tissue, the 10-mm subapical section would contain tissue similar to that in the normal 9-mm subapical section. Apical stem segments were excised and attached to index cards with two-sided adhesive tape and maintained in a vertical position in a humid, ethylene-free atmosphere. Sections were cut to the required 16-mm length at time zero and trimmed of the

[1] Michigan Agricultural Experiment Station Journal Article No. 8462.
[2] Present address: Department of Horticultural Science, Box 5216, North Carolina State University, Raleigh, North Carolina 27607.

apical and/or basal 3 mm at either zero time or after 15 min. The four treatments (apical 3 mm removed/basal 3 mm removed) were therefore tissue cut at zero/zero, zero/15 min, 15 min/zero, and 15 min/15 min. Twenty min after excision of the 16-mm subapical section, the 10-mm trimmed sections were transferred to 0.8-ml opaque glass sample chambers and the kinetic behavior of wound ethylene evolution was followed for 90 min.

The severity of wounding was studied by cutting 9-mm subapical sections into halves, thirds, fourths, or sixths. The tissue was placed in the sample chambers as soon as excised and subdivided, and the kinetics of ethylene evolution followed for 90 min.

Analysis of Data. Data from representative experiments were plotted to analyze the kinetics of wound-induced ethylene synthesis. A second degree polynomial was fitted to the data by the least squares method. All polynomials used had an r^2 of at least 0.99. The first derivative allowed calculation of the time and rate of maximum ethylene production. The integral allowed calculation of the amount of ethylene evolved during a 1-hr period centered about the time of maximum production. These parameters, similar data from accumulation studies, and other kinetic data were subjected to analysis of variance. Each experiment reported was repeated at least twice.

RESULTS AND DISCUSSION

Induction of Wound Ethylene Synthesis. Wound ethylene production by subapical sections excised from stems previously perturbed by shaking, flexing, or cutting had slightly different kinetics than sections excised from undisturbed seedlings. The maximum rate of wound ethylene synthesis occurred 56 min after excision of 9-mm subapical sections from freshly harvested 130-mm or 27-mm apical stem segments, or from 130-mm apical stem segments held in a humid, ethylene-free atmosphere for 15 min. Aging 27-mm apical stem segments for 15 min shortened the time to maximum ethylene synthesis by 5 min. The total amount of ethylene produced during the hr interval about the maximum was not significantly affected by any of the treatments. These data indicate that the cut used to prepare the 27-mm apical segment, from which the 9-mm subapical sections were excised, induced wound ethylene synthesis in the subapical region 9 mm away. If the excision of the 27-mm stem segment had not induced wound ethylene the maximum rate of wound ethylene synthesis would have occurred at 71 min (*i.e.*, 56 min + 15 min) rather than at the 51 min observed. The 5-min decrease in time to the maximum rate of production was statistically significant and indicates that the wound "signal" was not transmitted instantaneously; if it had been, the maximum would have occurred at (56 − 15 min =) 41 min. The observed transmission rate of 9 mm (5 min)$^{-1}$ (10.8 cm hr^{-1}) is within the velocity recorded for most transport phenomena in plants (2, 9).

Cutting subapical stem sections into apical and basal halves revealed that polarity of wound ethylene synthesis developed with time (Table I). Accumulation studies showed no significant difference in wound ethylene production by whole sections, sections cut in half, or the apical or basal halves during the 30-min lag period following excision. Basal halves produced 51% more wound ethylene than the apical halves during the 30- to 90-min collection interval. Ethylene production was the same for apical and basal halves during the 60- to 120-min collection interval. In the 90- to 150-min interval, the situation reversed so that the apical halves produced 49% more ethylene than the basal halves. Since sections excised near the apex produce more wound ethylene and exhibit prolonged high rates of production (15), the observed difference during the interval of the second peak (90–150 min) can readily be explained for the apical halves. However, production of more wound ethylene by basal halves during the first peak (30–90 min) is difficult to reconcile. Changes in the polarity of wound ethylene synthesis may indicate that necessary substrates accumulated at different rates at the two cut surfaces during the incubation period. This hypothesis was investigated in the next series of experiments.

Removal of apical and basal 3-mm stem sections from 16-mm subapical sections at zero time or at 15 min after the initial excision used to prepare the 16-min section did not significantly affect the time or rate of maximum wound ethylene synthesis, or the accumulation of wound ethylene in an hr interval about the maximum (Table II). This indicates that accumulation of a transportable substance(s) at the apical or basal cut surfaces did not account for the induction of, or significantly contribute to wound ethylene synthesis. Moreover, the wound stimulus was rapidly transmitted. If the excision cuts used to prepare the 16-mm subapical section were not sensed throughout the entire section, the time of maximum wound ethylene production for sections trimmed at zero time would have occurred at 56 min, while those trimmed at 15 min would have occurred at 71 min (56 min + 15 min). The times of maximum wound ethylene production differ by only about 1.9 min, showing that the induction began with the initial cut. The 9.5 cm hr^{-1} rate of wound signal transmission compares favorably with the 10.8 cm hr^{-1} rate calculated previously for subapical sections excised from 27-mm segments. The rate was calculated by dividing the length of time through which the signal traveled (3 mm) by the difference between times of maximum wound ethylene synthesis (1.9 min).

If cutting enhances ethylene production by the release of substance(s) from injured to adjacent cells, then additional wounding should further stimulate ethylene production as long as the zones of perturbed cells do not overlap. Cutting may therefore produce two zones of injured cells. Cells in zone I would be adjacent to the killed cells at the surface and would have a reduced rate of wound-induced ethylene synthesis. Cells in zone II, distant from the site of injury, would contain induced cells which would produce more ethylene per cell than in zone I. Cutting 9-mm subapical sections into small sections resolved the dimensions of these zones. Wound ethylene from whole sections or sections cut in half was about 0.043 nl mm^{-1} hr^{-1}. Cutting sections into thirds, fourths, or sixths reduced the rate of wound ethylene synthesis by about 30% to 0.029 ± 0.001 nl mm^{-1} hr^{-1}. These data imply that zone I must extend at least 1.5 mm, but not more than 2.25 mm, from the cut

Table I. Effect of cutting subapical stem sections into apical and basal halves on the production of wound ethylene.

Subapical Section	nl Ethylene g^{-1} hr^{-1}			
	Collection intervals (min from excision)			
	0-30	30-90	60-120	90-150
Apical half	4.2 a[1]	6.8 a	6.9 a	7.9 b
Basal half	3.8 a	10.3 b	7.2 a	5.3 a
Whole	3.7 a	---	---	---
Cut in half	3.6 a	---	---	---

[1]Means in the same column followed by the same letter are not significantly different at the 0.05 level.

Table II. Effect on wound ethylene synthesis of removing the apical and basal 3 mm from 16-mm subapical stem sections from etiolated 'Alaska' pea seedlings. The 16-mm section was excised 6 mm from the top of the apical hook. The 3-mm ends were removed at zero time or at 15 min after excision of the 16-mm section, and produced a 10-mm section of tissue from the same region of the pea stem as the normal 9-mm subapical section. The time and rate of maximum ethylene evolution were taken from the first derivative of a second degree polynomial fitted to the kinetic data by the method of least squares. The total ethylene evolved in a one-hr period centered about the maximum rate was calculated from the integral of the polynomial.

Time of cut (min)		Wound-induced ethylene synthesis		
Apical	Basal	Time of maximum (min)	Rate at maximum (nl g^{-1} hr^{-1})	Total Evolved (nl g^{-1})
0	0	56.6 ± 0.8[1]	15.1 ± 2.3	5.2 ± 0.7
0	15	56.7 ± 1.9	14.1 ± 1.9	5.1 ± 0.5
15	0	57.2 ± 0.2	13.8 ± 1.8	5.0 ± 0.5
15	15	58.5 ± 1.3	12.4 ± 3.3	4.9 ± 0.8

[1]Means plus or minus the standard deviation.

surface. Excision of the subapical section induces wound ethylene throughout the entire section, otherwise additional wounding would increase wound ethylene synthesis by inducing more cells; yet additional wounding decreased the rate of wound-induced ethylene synthesis.

The wound response is complex. Cutting subapical sections into smaller segments or removal of strips of epidermis not only decreased the maximum rate of wound ethylene production, but also delayed the onset of the response. The normal lag of 26 min was increased to 30 min for peeled subapical sections, and to 36 min for subapical sections cut into 2-mm sections. Both treatments reduced maximum rates of ethylene production by about 30%. The increased lag period may imply that it is a phase during which perturbed cells recover from the initial effect of trauma.

The actual mechanism by which cells are informed of a wound made several cm away remains an intriguing problem. The velocity with which the signal is transmitted suggests that the messenger could be a translocated chemical, or a physical or nerve-like stimulus. Wounding has been shown to cause hyperpolarization of cells within about 30 min (7, 11).

LITERATURE CITED

1. ABELES AL, FB ABELES 1972 Biochemical pathways of stress-induced ethylene. Plant Physiol 50: 446–498
2. CANNY MJ 1971 Translocation: mechanisms and kinetics. Annu Rev Plant 22: 237–260
3. ELSTNER EF, JR KONZE 1976 Effect of point freezing on ethylene and ethane production by sugar beet leaf discs. Nature 263: 351–352
4. IMASEKI, H, I URITANI, MA STAHMANN 1968 Production of ethylene by injured sweet potato root tissue. Plant Cell Physiol 9: 757–768
5. IMASEKI H, C PJON, M FURUYA 1971 Phytochrome action in *Oryza sativa* L. IV. Red and far red reversible effects on the production of ethylene in excised coleoptiles. Plant Physiol 48: 241–244
6. JACKSON MB, DJ OSBORNE 1970 Ethylene, the natural regulator of leaf abscission. Nature 225: 1019–1022
7. KOOPOWITZ H, R DHYSE, DE FOSKET 1975 Cell membrane potentials of higher plants: changes induced by wounding. J Exp Bot 26: 131–137
8. LATIES GG 1962 Controlling influence of thickness on development and type of respiratory activity in potato slices. Plant Physiol 37: 679–690
9. LÄUCHLI A 1972 Translocation of inorganic solutes. Annu Rev Plant Physiol 23: 197–218
10. LIEBERMAN M, AT KUNISHI 1971 Synthesis and biosynthesis of ethylene. HortScience 6: 355–358
11. MACKLON AES, N HIGINBOTHAM 1968 Potassium and nitrate uptake and cell transmembrane electropotential in excised pea epicotyls. Plant Physiol 43: 888–892
12. MCGLASSON WB 1969 Ethylene production by slices of green banana fruit and potato tuber tissue during the development of induced respiration. Aust J Biol Sci 22: 489–491
13. MEIGH DF, KH NORRIS, CC CRAFT, M LIEBERMAN 1960 Ethylene production by tomato and apple fruits. Nature 186: 902–903
14. NAKAGAKI Y, T HIRAI, MA STAHMANN 1970 Ethylene production by detached leaves infected with tobacco mosaic virus. Virology 40: 1–9
15. SALTVEIT ME JR, DR DILLEY 1978 Rapidly induced wound ethylene from excised segments of etiolated *Pisum sativum* L., cv. Alaska. I. Characterization of the response. Plant Physiol 61: 447–450
16. SALTVEIT ME JR, DR DILLEY 1978 Rapidly induced wound ethylene from excised segments of etiolated *Pisum sativum* L., cv. Alaska. II. Oxygen and temperature dependency. Plant Physiol 61: 675–679
17. WRIGHT STC 1976 The relationship between leaf water potential and the levels of ABA and ethylene in excised wheat leaves. *In* PE Pilot, ed, 9th International Conf on Plant Growth Substances, Lausanne, pp. 436–438