# Ethylene as an Effector of Wound-Induced Resistance to Cellulase in Oat Leaves[1]

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GORDON T. GEBALLE[2] AND ARTHUR W. GALSTON
*Department of Biology, Yale University, New Haven, Connecticut 06511*

## ABSTRACT

**Peeling the abaxial epidermis from oat leaves (*Avena sativa* var. Victory) induces the formation of wound ethylene and the development of resistance to cellulolytic digestion of mesophyll cell walls. Ethylene release begins between 1 and 2 hours after peeling in the light or dark. Aminoethoxyvinylglycine (AVG, 0.1 millimolar), $CoCl_2$ (1.0 millimolar), propyl gallate (PG, 1.0 millimolar) or aminooxyacetic acid (AOA, 1.0 millimolar) inhibits, whereas $AgNO_3$ stimulates wound ethylene formation. Incubation on inhibitors of ethylene biosynthesis (AVG, $CoCl_2$, PG, AOA) or action ($AgNO_3$, hypobaric pressure or the trapping of ethylene with $HgClO_4$) also prevents the development of wound-induced resistance to enzymic cell wall digestion. 1-Aminocyclopropane-1-carboxylic acid (ACC, 1.0 millimolar) reverses AVG (0.1 millimolar) inhibition of the development of resistance. Exogenous ethylene partially induces the development of resistance in unwounded oat leaves.**

**These results suggest that peeling of oat leaves induces ethylene biosynthesis, which in turn effects changes in the mesophyll cells resulting in the development of resistance to cellulolytic digestion.**

Many stresses, including fungal (23, 27), bacterial (19), or viral (8) infection, waterlogging, drought and noxious chemicals (1, 24) induce ethylene biosynthesis in plants. Wounding, a common event in the field, induces ethylene release in green figs (10, 31), tomatoes (6, 22), green bananas (21), bean leaves (15), etiolated pea (25), and mandarin orange (13).

With the identification of 1-aminocylopropane-1-carboxylic acid as an intermediate (2), the proposed biosynthetic pathway for ethylene, Met[3] → SAM → ACC → ethylene, is being studied extensively; for instance, the wounding of tomato pericarp tissue induces ACC synthase and ACC synthesis (6). Inhibitors have been used to study the steps in this pathway. AVG (30) and AOA (4) inhibit the conversion of SAM to ACC, whereas $CoCl_2$ (16, 30) and PG (18) inhibit the conversion of ACC to ethylene. $AgNO_3$ does not inhibit ethylene synthesis, but interferes with its action (5). Hypobaric pressure (7) and $HgClO_4$ (29) inhibit ethylene action by lowering the internal concentration of the hormone.

This paper reports experiments designed to study the effect of peeling on the biosynthesis of wound ethylene by oat leaves, and to test the hypothesis that wound-induced ethylene is the effector of the development of wound-induced resistance to cellulase (12).

## MATERIALS AND METHODS

Oat seedlings (*Avena sativa* var. Victory) were grown, peeled, and digested with Cellulysin (Calbiochem, 0.5%, 0.6 M mannitol [pH 5.6] at 31°C) as described in the previous paper (12). Peeled leaf segments were floated on test solutions for 24 h, then digested on Cellulysin. Although Cellulysin contains other enzymic activities, *e.g.* pectinolytic and proteolytic, experiments with a more purified cellulase suggest that it is the cellulolytic component which causes digestion (12).

Gas chromatography was used to detect and measure ethylene release by peeled and unpeeled oat leaves. Tissue (7 × 5 cm long leaf segments) was incubated in the dark on test solution (5 ml) in a glass bottle sealed with a rubber stopper (final gas volume = 71.3 ± 2.0 ml), and the internal pressure was equilibrated to atmospheric pressure by inserting and removing a syringe needle through the stopper. The bottles were incubated on their sides to facilitate contact between the tissue and the medium.

After 24 h, a gas sample (5 ml) was removed with a gas tight syringe and injected into a Perkin-Elmer model F-11 gas chromatograph equipped with a flame ionization detector. Nitrogen carried the sample through a 2-m stainless steel column packed with activated alumina at room temperature. Ethylene was identified by comparing the retention times of unknown peaks with that of ethylene standards (Fisher) and the removal of the presumed ethylene peak by $HgClO_4$, and was quantified by comparing peak heights with those of known amounts of ethylene.

The effect of exogenous ethylene on unwounded oat leaves was tested by placing detached leaf segments in beakers of distilled $H_2O$ into desiccators of known volumes. After the desiccators were sealed, internal pressure was decreased slightly by aspiration; then ethylene was injected into the entering air stream (final concentration of ethylene = 100 μl/l, 10 μl/l, 1 μl/l, 0.1 μl/l, or 0.0 μl/l). After 24 h, the tissue was removed, peeled and exposed to Cellulysin. The air control contained a separate beaker of $HgClO_4$ to adsorb any ethylene in the air, and each desiccator contained a beaker of fresh, saturated KOH to trap $CO_2$, an inhibitor of ethylene action (18, 26). These experiments were conducted in room light and at room temperature.

For all experiments the mean of at least three separate experiments is reported.

## RESULTS AND DISCUSSION

Table I shows that unpeeled leaf segments produce ethylene, possibly due to the cuts necessary for separating the tissue from the leaf; alternatively, unwounded tissue may normally make a small amount of ethylene (18). Peeling induces a 4-fold increase

[1] This work was submitted as partial fulfillment for the Ph.D. degree, Yale University.

[2] Present address: School of Forestry and Environmental Studies, Yale University, New Haven, CT 06511; to whom correspondence should be addressed.

[3] Abbreviations: Met, methionine; SAM, *S*-adenosylmethionine; ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycine; AOA, aminooxyacetic acid; AOPP, aminooxyphenylpropionic acid; PG, propyl gallic acid; AD, actinomycin D; CH, cycloheximide.

Table I. *Effect of Inhibitors on Ethylene Release and Development of Resistance to Cellulysin*

Segments were floated on test solutions for 24 h in the dark at 24.5°C, except $HgClO_4$, which was in a separate vial. Hypobaric pressure = 0.4 atm.

| Treatment | Ethylene Release | Digestion |
|---|---|---|
| | *nl/g fresh wt* | *% fresh* |
| Unpeeled | 6.9 | 100 |
| Peeled | 28.4 | 0 |
| on AVG (100 μM) | 1.9 | 100 |
| on AVG (10 μM) | 6.3 | 48 |
| on $CoCl_2$ (1 mM) | 2.5 | 95 |
| on $CoCl_2$ (0.1 mM) | 4.4 | 97 |
| on PG (10 mM) | 3.2 | 100 |
| on PG (1 mM) | 17.6 | 100 |
| on AOA (1 mM) | 1.9 | 82 |
| on AOA (0.1 mM) | 8.8 | 58 |
| on AOPP (1 mM) | 17.6 | 100 |
| on AOPP (0.1 mM) | 28.4 | 80 |
| on AD (20 μg/ml) | 31.5 | 90 |
| on AD (10 μg/ml) | 25.2 | 15 |
| on CH (1 μg/ml) | 9.5 | 100 |
| on CH (0.1 μg/ml) | 12.6 | 67 |
| +$HgClO_4$ | 1.9 | 82 |
| +Hypobaric | | 95 |

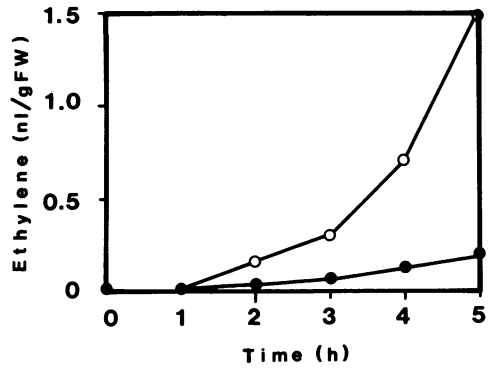


FIG. 1. Time course of ethylene release following peeling. Bottles containing either unpeeled (●) or peeled (○) segments were sealed and samples were removed hourly with only one sample taken from each bottle.

Table II. *ACC-Reversal of AVG (0.1 mM) -Inhibition of the Development of Resistance to Cellulysin*

Segments were floated on AVG + ACC for 24 h in the dark at 24.5°C.

| ACC Concn. | Digestion |
|---|---|
| *mM* | *% fresh* |
| 1.0 | 6 |
| 0.1 | 51 |
| 0.01 | 99 |
| 0.0 | 98 |

in ethylene synthesis and the development of resistance to digestion by Cellulysin (Table I). Figure 1 shows that ethylene release from wounded tissue begins between the 1st and 2nd h after wounding and continues for at least 5 h.

AVG and $CoCl_2$ prevent the synthesis of ethylene and also inhibit the development of resistance to Cellulysin (Table I). AVG, effective at submillimolar concentrations, is probably not acting as a protein synthesis inhibitor (20). When ACC (1.0 mM) is added with AVG (0.1 mM) resistance develops (Table II). Although $AgNO_3$ (0.3 mM) stimulates ethylene synthesis 4-fold

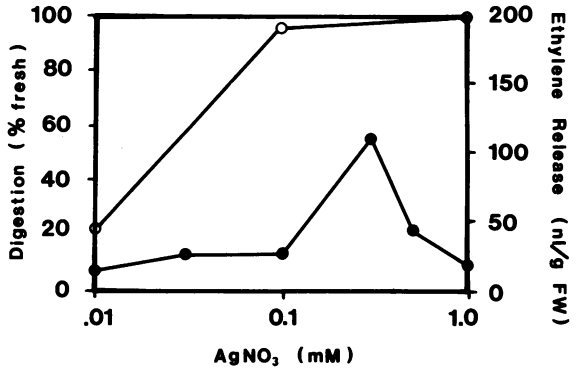


FIG. 2. Effect of $AgNO_3$ on ethylene release (●) and development of resistance to Cellulysin (○).

Table III. *Effect of Exogenous Ethylene on Unpeeled Oat Leaves*

| Treatment | Digestion |
|---|---|
| | *% fresh* |
| Fresh | 100 |
| Ethylene, μl/l[a] | |
| 0.0 | 102 |
| 0.1 | 109 |
| 1.0 | 73 |
| 10.0 | 42 |
| 100.0 | 41 |

[a] All treatments contained beakers of KOH to trap $CO_2$.

over wounding and 16-fold over unwounded tissue (Fig. 2), it prevents the development of resistance (Fig. 2), reinforcing the hypothesis that silver ion blocks ethylene action (5), regardless of any effect on synthesis. At concentrations greater than 1 mM, $AgNO_3$-treated tissue is flaccid and ethylene synthesis and the development of resistance are inhibited; presumably, the silver ion is toxic to the tissue. At concentrations less than 0.1 mM, ethylene synthesis is unaffected by the presence of silver ion and resistance develops at 0.01 mM $AgNO_3$. This stimulation of ethylene synthesis by silver ion, also found in tobacco leaf discs (3), is similar to that caused by other metal ions, *e.g.* $Cu^{2+}$ (17) and $Cd^{2+}$ (9), possibly indicating the stress caused by these metals.

PG (10 mM) inhibits ethylene synthesis and the development of resistance to Cellulysin (Table I). At 1 mM, PG inhibits the increase in ethylene synthesis by only 50%, but completely inhibits the development of resistance, suggesting that PG interferes with another step in the development of the wound response, probably wound-induced lignification (11).

AOA (1 mM) inhibits both ethylene synthesis and the development of resistance (Table I). AOPP, although similar chemically, acts differently; it does not affect ethylene synthesis but inhibits the development of resistance (Table I) by inhibiting phenylalanine ammonia lyase activity (11).

AD (20 μg/ml) inhibits the development of resistance but has no effect on ethylene biosynthesis (Table I), whereas CH (1 μg/ml) inhibits both processes. It is not clear from these results whether RNA synthesis is required for wound-induced ethylene synthesis. It is possible that AD does not penetrate the tissue quickly enough to show an inhibition or that only protein synthesis is required for wound-induced ethylene formation. Both RNA and protein synthesis appear to be required for the development of resistance (12).

Removing ethylene from the air with $HgClO_4$ prevents the development of resistance (Table I). Hypobaric pressure (0.4 atm) also prevents the development of resistance (Table I). These two methods of inhibiting the development of resistance probably act by lowering the internal concentrations of ethylene (7, 29).

Exogenous ethylene partially induces the development of re-

sistance in unpeeled tissue (Table III). The concentration for threshold (1 μl/l) and half-maximal (10 μl/l) responses are similar to those of other ethylene-mediated responses (1). Similarly, Weinbaum *et al.* (28) have reported that prune mesocarp tissue becomes partially resistant to digestion by Cellulysin and macerase when treated with ethylene. Possible reasons for the failure of exogenous ethylene to substitute completely for wounding include the following. (a) Because of the presence of the epidermis (these are unpeeled leaf segments) internal ethylene concentration may not reach the level caused by wounding. (b) Exogenous ethylene may not affect as many cells as does wounding. (c) Peeling the epidermis, required for digestion, may disturb the mesophyll cell walls in such a way that the cause of the resistance is disturbed. (d) The induction of resistance to cellulase may require a wound signal in addition to ethylene.

The results of this study show that peeling causes oat leaves to release four times as much ethylene as unpeeled leaves (Table I) and that the ethylene, in turn, induces the development of resistance to cellulolytic digestion of mesophyll cell walls. When ethylene synthesis is blocked by inhibitors, then the development of resistance is also inhibited (Table I). ACC, the immediate precursor of ethylene, reverses inhibition by AVG of the development of resistance (Table II). The rate of ethylene release increases approximately 1 h after wounding (Fig. 1). Although this increase is not detected as early as in other systems (14, 15, 25), it occurs before resistance begins to develop. The wounded leaf could provide a useful system for studying the biosynthesis and mode of action of the plant hormone ethylene.

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