Plant Physiol. (1972) 50, 496–498

Biochemical Pathway of Stress-induced Ethylene

Received for publication March 28, 1972

A. L. ABELES[1]
Science Department, Frederick Community College, Frederick, Maryland 21701

F. B. ABELES
Plant Air Pollution Laboratory, Plant Science Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705

ABSTRACT

Ethylene production from bean and tobacco leaves increased rapidly following the application of toxic compounds such as $CuSO_4$, Endothal, and ozone. Treatments which increased ethylene evolution also increased the conversion of U-^{14}C-methionine into ethylene. Cycloheximide inhibited the production of chemical stress-induced ethylene. These results suggest that ethylene is produced by the same biochemical pathway forming basal ethylene, auxin-induced ethylene, or that produced during the ripening of climacteric fruit.

Ethylene production in plants often increases following damage, wounding, or stress from a variety of sources. This increase has been called wound ethylene, but a better term might be stress-induced ethylene production, or, more simply, stress ethylene. Williamson (18) was the first to point out that injured cells often produced more ethylene than normal tissue. A significant body of literature indicates that the causes of stress ethylene are varied and include chemicals (5), insects (7), temperature extremes (17), drought (13), γ-irradiation (17), disease (16), and mechanical effects such as incision (10), pressure (9), and abrasion (5). The function of stress ethylene may be to cause abscission of damaged plant organs. It may also play a regulatory role in the growth of seedlings through soil (9) and be a part of disease resistance mechanisms (16). It is important to point out that stress ethylene is a product of living tissue, since it ceases when damage is severe enough to kill.

Recent research (2) supports the idea that endogenous basal ethylene, auxin-induced ethylene, and ethylene from ripening fruits comes from methionine. The work described here was designed to determine if stress ethylene was produced by the same pathway and if methionine was the source.

MATERIALS AND METHODS

Beans (*Phaseolus vulgaris* L., cv. Red Kidney) and tobacco (*Nicotiana tabacum* L., cv. Xanthi) were grown in soil in a greenhouse. Primary leaves from 2-week-old beans and 100-cm leaves from 6-week-old tobacco plants were excised and placed in tubes containing unlabeled or U-^{14}C-methionine. The leaves were placed in a hood for an hour, and the methionine was drawn up by transpiration. All of the methionine supplied to the leaves was taken up and transported to the blade. The amount and specific radioactivity of methionine used in each experiment is indicated in Table I. Leaves were sprayed to run off with 2.5% $CuSO_4$, 0.06% Endothal (3,6-endoxohexahydrophthalic acid), plus 1% Tween-20 surfactant or 1 mM naphthaleneacetic acid (NAA)[2] to increase ethylene production. A 1-hr, 0.5 μl/liter, ozone fumigation was used to promote ethylene production from tobacco.

Each experiment consisted of four 3-liter desiccators containing six leaves with their petioles in a solution of 1 mM unlabeled methionine, and a dish with filter paper soaked with 1 ml of 20% KOH (w/v) to absorb CO_2. Desiccator 1 held unlabeled leaves sprayed with water. Desiccator 2 held unlabeled leaves sprayed with the test chemical. Rates of ethylene production were determined by withdrawing 2-ml samples with a syringe through a vaccine stopper on the side arm of the lid collar and injecting them into a gas chromatograph. Desiccator 3 held labeled leaves sprayed with water and a dish containing 5 ml of 0.25 M mercuric perchlorate in 4 M perchloric acid to absorb ^{14}C-ethylene. Desiccator 4 held labeled leaves sprayed with the test chemical and a mercuric perchlorate trap. A slight vacuum was applied to the desiccators to prevent the lids from slipping, and desiccators 3 and 4 were placed on a reciprocal shaker to facilitate uptake of ^{14}C-ethylene into the mercuric perchlorate. Ethylene accumulation in desiccators 1 and 2 was measured by gas chromatography every hour for 3 to 5 hr. At the end of an experiment, 4.5 ml of mercuric perchlorate were transferred from the desiccators to 10-ml tubes sealed with rubber vaccine stoppers. The test tubes were placed in crushed ice and evacuated by inserting a syringe needle, attached to a vacuum pump, through the rubber stopper. ^{14}C-Ethylene was released by injecting 4 ml of 4 M LiCl into each tube and heating it to 80 C on a water bath. The labeled ethylene was then transferred to evacuated scintillation vials, sealed with rubber stoppers, containing 1 ml of 0.1 M mercuric acetate in methanol. The gas phase in the test tube was withdrawn with a 5-ml gas-tight disposable syringe and injected into the scintillation vial five times. The vials were then placed in crushed ice and shaken occasionally in a 30-min period to absorb the ethylene. Fourteen milliliters of scintillation fluid (90 ml of toluene, 90 ml of ethylene glycol monoethyl ether, 90 ml of dioxane, 12.6 ml of Liquifluor (to give 0.4% PPO and 0.05% POPOP), and 30 g of naphthalene) was

[1] This work was performed when A. L. Abeles was on a summer program for teachers, sponsored by the Educational Services Branch, Information Division, United States Department of Agriculture, Beltsville, Md.

[2] Abbreviation: NAA: naphthaleneacetic acid.

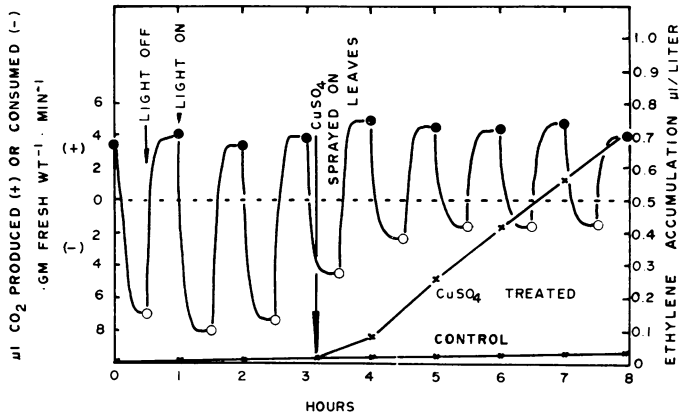

FIG. 1. Effect of 2.5% $CuSO_4$ on ethylene production, respiration, and photosynthesis of bean leaves.

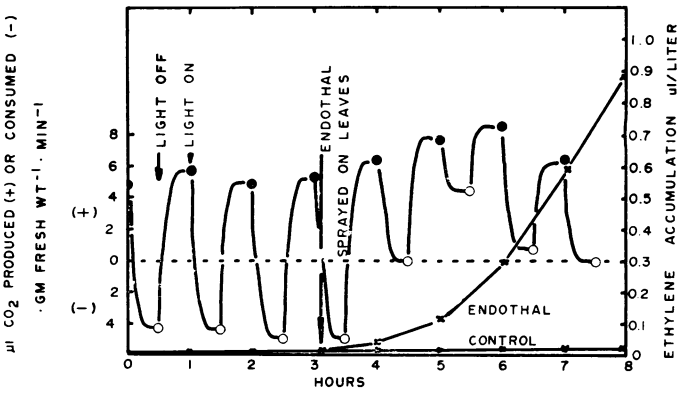

FIG. 2. Effect of 0.06% Endothal on ethylene production, respiration, and photosynthesis of bean leaves.

Table I. *Effect of $CuSO_4$, Endothal, NAA, and Ozone on Ethylene Production and Conversion of U-^{14}C-Methionine into Ethylene*

Concentrations were: $CuSO_4$: 2.5%; Endothal: 0.06% with 1% Tween-20; NAA: mM, ozone: 0.5 µl/liter for 1 hr. Experiments 1 through 3 were with bean leaves, and number 4 was with tobacco. For experiments 1 and 2, specific radioactivity was 222 c/mole; for 3 and 4, 15 c/mole. Calculations were based on the assumption that 40% of the methionine was converted into ethylene.

Expt. No.	Treatment	Duration of Experiment	Ethylene Produced	^{14}C-Ethylene Produced	Specific Radioactivity	Conversion Efficiency[1]
		hr	*nmoles*	*nc*	*ci/mole*	%
1	Control	5	7.55	3.68	0.49	0.55
	$CuSO_4$		130.0	37.6	0.29	0.33
2	Control	3	5.05	4.93	0.98	1.1
	Endothal		99.6	49.8	0.50	0.5
3	Control	5	2.58	0.13	0.05	0.81
	NAA		16.4	0.50	0.03	0.48
4	Control	3.5	4.46	0.016	0.0036	0.06
	Ozone		31.0	0.066	0.0021	0.03

[1] Specific radioactivity ^{14}C-ethylene produced-specific radioactivity U-^{14}C-methionine used.

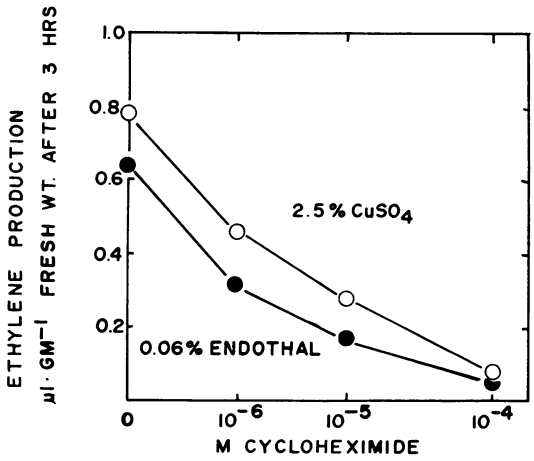

FIG. 3. Effect of cycloheximide on the formation of stress ethylene induced by $CuSO_4$ and Endothal. Leaves were infiltrated with cycloheximide and then sprayed with the chemicals shown. Ethylene production from leaves placed in 125-ml Erlenmeyer flasks was measured after 3 hr.

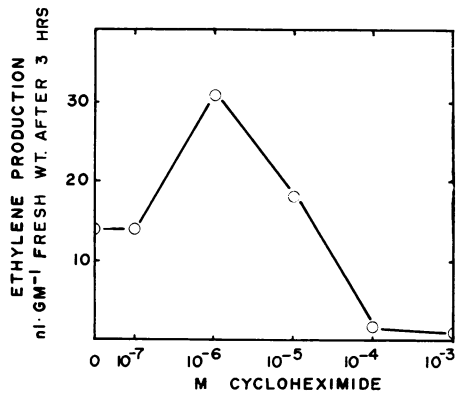

FIG. 4. Effect of cycloheximide on ethylene production from bean leaves. Leaves were infiltrated with cycloheximide for 1 hr and placed in 125-ml Erlenmeyer flasks. Ethylene production was measured 3 hr later.

added and the radioactivity of the vials assayed in a scintillation counter. Preliminary tests demonstrated that 90% or more of the ethylene trapped in the mercuric perchlorate was transferred to the scintillation vials using this method.

Photosynthesis and respiration were measured by monitoring the CO_2 content of air leaving a 2-liter glass reaction vessel with an infrared CO_2 gas analyzer. A 50-ml Erlenmeyer flask containing either six 10-day-old bean seedlings excised at the cotyledonary node or 12 g of tobacco leaves were placed in the reaction vessel on a perforated support raised 3 cm from the bottom of the vessel. Air was passed through the reaction vessel at a rate of 1 liter per min, and a magnetic stirring bar was placed below the perforated plate to facilitate equilibration of the gas phase.

The apparatus was placed in a darkened room and a clock-operated 150-w incandescent lamp placed 10 cm from the vessel. The lamp was turned on and off at 30-min intervals. The difference between the level of CO_2 entering the vessel and that leaving it was used to estimate respiration and photosynthesis. Three hours after the start of the experiment the leaves were removed and sprayed with the indicated solutions. Ethylene production during the course of these experiments was determined by placing an equivalent sample of leaves in a 3-liter desiccator and sampling the gas phase at 1-hr intervals.

Dose response curves for the effect of cycloheximide on ethylene production by leaves treated with $CuSO_4$ and Endothal were measured by infiltrating bean leaves with various concentrations of cycloheximide as described above. Leaves were then sprayed with $CuSO_4$ and Endothal and placed in 125-ml Erlenmeyer flasks sealed with rubber vaccine stoppers. Samples were withdrawn and measured by gas chromatography at 1-hr intervals.

RESULTS

The effect of $CuSO_4$ and Endothal on ethylene production, respiration, and photosynthesis is shown in Figures 1 and 2. Dose response curves had established that these concentrations gave a maximal effect. These chemicals cause a rapid rise in ethylene production and simultaneously inhibit photosynthesis and, in the case of Endothal, increase respiration. Similar experiments with ozone on tobacco leaves failed to show any effect on either photosynthesis or respiration.

The conversion of methionine to ethylene in bean and tobacco leaves is shown in Table I. The conversion efficiency for the transfer of carbons 3 and 4 into ethylene was about 0.8% for beans and 0.06% for tobacco. $CuSO_4$ and Endothal increased ethylene production about 20-fold and ^{14}C-ethylene formation 10-fold. Ozone increased ethylene production 9-fold and also reduced the conversion efficiency 50%. NAA also increased ethylene production and the conversion of methionine into ethylene.

The effect of $CuSO_4$ and Endothal on ethylene production and the ability of cycloheximide to decrease it is shown in Figure 3. Low concentrations of cycloheximide consistently doubled ethylene production, but the effect was lost as the concentration used was increased (Fig. 4). Cycloheximide does not appear to be toxic since we found that it had no effect on photosynthesis or respiration at the concentrations used.

DISCUSSION

Ethylene production increases during ripening, as a result of auxin stimulation and as a result of stress. Lieberman and Mapson (11) were the first to suggest that methionine was the source of ethylene in plants. Burg and Clagett (4) reported that the conversion of methionine to ethylene was low and beyond the limits of detection unless IAA was present, and Baur *et al.* (3) observed conversion of methionine to ethylene in climacteric avocado, but not in preclimacteric fruit. We found that endogenous ethylene produced by leaves came from methionine, and stress ethylene, induced by toxic compounds, was also produced by methionine. However, the data show that the percentage conversion efficiency fell 50% during the production of stress ethylene. This was due either to the operation of another biochemical pathway or the fact that more unlabeled endogenous methionine was being utilized when the rate of ethylene production increased.

Ethylene production associated with hormones can be blocked with inhibitors of RNA and protein synthesis (1) including cycloheximide (15). Ethylene production from ripening fruit was also blocked with cycloheximide (8). The data presented in Figure 3 demonstrated that stress ethylene production was blocked with cycloheximide, and the stress ethylene induced by low concentrations of cycloheximide was blocked as the level of inhibitor was raised (Fig. 4).

The data presented here suggest that biochemistry of stress ethylene is similar, if not identical, to ethylene production during ripening and application of hormones. The activation of the system was rapid, since differences between control and treated tissues were apparent 50 min after the application of the chemicals and seemed to require protein synthesis. Confirmation on this point requires identification of the enzymes involved in the conversion of methionine to ethylene.

Plants seem to have a system capable of responding to sublethal trauma, and stress ethylene production appears to be a part of it. Whether or not ethylene plays a role in repair or defense mechanisms, abscission of damaged organs, or is simply symptomatic of cellular disorganization remains to be clarified.

LITERATURE CITED

1. Abeles, F. B. 1966. Auxin stimulation of ethylene evolution. Plant Physiol. 41: 585–588.
2. Abeles, F. B. 1972. The biochemistry and mode of action of ethylene. Annu. Rev. Plant Physiol. 23: 259–292.
3. Baur, A. H., S. F. Yang, H. K. Pratt, and J. B. Biale. 1971. Ethylene biosynthesis in fruit tissue. Plant Physiol. 47: 696–699.
4. Burg, S. P. and C. O. Clagett. 1967. Conversion of methionine to ethylene in vegetative tissue of fruits. Biochem. Biophys. Res. Commun. 27: 125–130.
5. Cooper, W. C., G. K. Rasmussen, B. J. Rogers, P. C. Reece, and W. H. Henry. 1968. Control of abscission in agricultural crops and its physiological basis. Plant Physiol. 43: 1560–1576.
6. Craker, L. 1971. Ethylene production from ozone-injured plants. Environ. Pollut. 1: 299–304.
7. Galil, J. 1968. An ancient technique for ripening sycamore fruit in East-Mediterranean countries. Econ. Bot. 22: 178–190.
8. Frenkel, C., I. Klein, and D. R. Dilley. 1968. Protein synthesis in relation to ripening of pome fruits. Plant Physiol. 43: 1146–1153.
9. Goeschl, J. D., L. Rappaport, and H. K. Pratt. 1966. Ethylene as a factor regulating the growth of pea epicotyls subjected to physical stress. Plant Physiol. 41: 877–889.
10. Hall, W. C. 1951. Studies on the origin of ethylene from plant tissues. Bot. Gaz. 113: 55–65.
11. Lieberman, M. and L. W. Mapson. 1964. Genesis and biogenesis of ethylene. Nature 204: 343–345.
12. Maxie, E. C. and A. Abdel-Kader. 1966. Food irradiation of fruits as related to feasibility of the technology. Advan. Food Res. 15: 105–145.
13. McMichael, B. L., W. R. Jordon, and R. D. Powell. 1972. An effect of water stress on ethylene production by intact cotton petioles. Plant Physiol. In press.
14. Ross, A. F. and C. E. Williamson. 1951. Physiologically active emanations from viral infected plants. Phytopathology 41: 431–438.
15. Sakai, S. and H. Imaseki. 1971. Auxin-induced ethylene production by mung bean hypocotyl segments. Plant Cell Physiol. 12: 349–359.
16. Stahmann, M. A., B. G. Clare, and W. Woodbury. 1966. Increased disease resistance and enzyme activity induced by ethylene and ethylene production by black rot infected sweet potato tissue. Plant Physiol. 41: 1505–1512.
17. Vines, H. M., W. Grierson, and G. J. Edwards. 1968. Respiration, internal atmosphere, and ethylene evolution of citrus fruit. Proc. Amer. Soc. Hort. Sci. 92: 227–234.
18. Williamson, C. E. 1950. Ethylene, a metabolic product of diseased or injured plants. Phytopathology 40: 205–208.