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Abscission: The Role of Ethylene, Ethylene Analogues, Carbon Dioxide, and Oxygen

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Abstract. **Ethylene was the most effective abscission accelerant examined, with decreasing activity shown by propene, carbon monoxide, acetylene, vinyl fluoride, 1-butene, and 1,3-butadiene. Carbon dioxide inhibited abscission, but its effect was overcome by ethylene. Oxygen was required for abscission as an electron acceptor for respiration and not as a potentiator or activator of the ethylene attachment site. The molecular requirements for abscission were similar to those shown by other workers for other biological processes under the influence of ethylene.**

Practically all plants grow and develop in the presence of small amount of ethylene. However, any potential plant response depends on the levels of ethylene present and the susceptability of cells to ethylene action. This gaseous hormone influences a number of processes, including abscission, bulb dormancy, celery blanching, epinasty, fruit ripening, flowering, flower fading, growth inhibition, induction of peroxidase, and seed germination (8). The characterization of the attachment site of this simple 2-carbon molecule in the cell is a part of the problem of how ethylene regulates plant growth and development. If the ethylene attachment site were similar for all of the above processes, the initial reaction of different kinds of cells to the gas probably would also be similar. A number of initial reactions to ethylene presently being considered include alteration of membrane permeability (18), regulation of auxin activity (10, 21), and regulation of nucleic acid metabolism (16).

The ethylene attachment site has been characterized by (i) comparing the relative effectiveness of ethylene and its analogues, (ii) indicating that carbon dioxide acts as a competitive inhibitor, and (iii) determining if the activity of ethylene is influenced by oxygen under conditions where the role of oxygen as an electron acceptor for respiration can be ignored or accounted for. Such a comparison was made for the role of ethylene in growth inhibition and fruit ripening by Burg and Burg (9).

The relative effectiveness of ethylene and other unsaturated gases in abscission proved similar to that reported earlier for growth inhibition, epinasty, and fruit ripening. In agreement with Yamaguchi (26), we found that abscission was inhibited by carbon dioxide and that ethylene would overcome the inhibition. However, the effect of oxygen on abscission appeared to be due to its effect on respiration.

Materials and Methods

The abscission-regulating activity of unsaturated gases was determined by adding them to the gas phase above bean (*Phaseolus vulgaris* L. cv. Red Kidney) explants aged 21 hours at 25° in gas collection bottles. The methods used to grow bean plants and to prepare and store abscission zone explants were described earlier (2, 3, 4). The high levels of ethylene production from freshly excised explants which may represent wound ethylene was removed 5 hours after the explants were excised by flushing the gas collection bottles with air. The bottles remained sealed for an additional 16 hours and were then flushed again before adding the gases to be tested. Ethylene contamination of the gases used was determined by gas chromatography (4) and found to be below levels that would interfere with their use.

Coleus (*Coleus blumei* Benth.), cotton (*Gossypium hirsutum* L., cv. Acala 4-42) and *Cassia* (*Cassia fistula* L.) explants were prepared as described earlier (1). Carbon dioxide was added to the gas phase surrounding the explants immediately after excision. The carbon dioxide was removed from the control atmospheres by placing a vial containing 10 % KOH and a filter-paper wick in the gas collection bottles. The bottles were vented 24 hours after the start of the experiment, resealed, and carbon dioxide reinjected. Abscission was measured at 48 hours. Ethylene contamination of the carbon dioxide was less than 0.01 ppm.

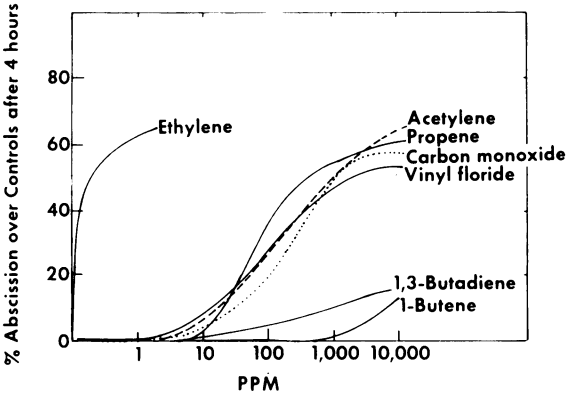
The reversal of carbon dioxide inhibition by ethylene was measured by adding various concentrations of carbon dioxide and ethylene to the gas collection bottles 21 hours after the explants were excised. Abscission was measured 4 hours later. Wound ethylene was removed by venting the bottles 5 hours after excision.

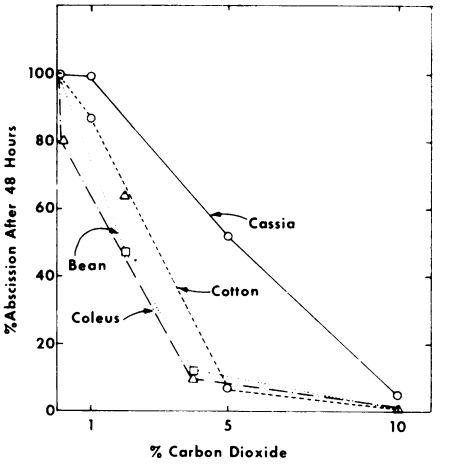
The effect of oxygen on oxygen uptake by bean explants was measured by using Warburg manometric techniques. Explants were stored for 24 hours in petri dishes containing plain agar and then placed in 2 ml of 1 % agar in the bottom of the Warburg flasks. Oxygen-nitrogen mixtures were

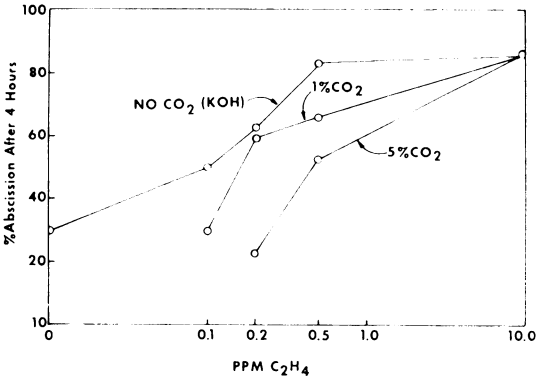
prepared from commercial gases and stored under pressure in a steel container fitted with appropriate control valves. Oxygen concentration in the gas mixture was determined by comparing peak heights on a gas chromatograph (7) with those obtained from carefully prepared mixtures of the pure gases. Respiration was measured in the absence of carbon dioxide at 25° with stationary manometers. The system was diffusion-limited at low oxygen tensions, but the conditions were similar to those experienced by explants in oxygen experiments and gave an estimation of the rate of respiration measured as oxygen consumption. The effect of oxygen on abscission was measured by storing explants in gas collection bottles covered with cheesecloth for 19 hours. The bottles were then sealed with rubber vaccine caps, the gas phase flushed with nitrogen, and 0.1 ppm ethylene and appropriate amounts of oxygen added. Abscission was measured 4 hours later.

Results

Ethylene and Ethylene Analogues. The effects of ethylene and other gases on abscission are shown in figure 1. Half-maximum stimulation of abscission occurred at about 0.1 ppm ethylene. All of the other gases tested showed less activity. Burg and Burg (9) also observed that 0.1 ppm ethylene was half-maximum in inhibiting pea stem growth. A comparison of gases based on the concentrations required for half-maximum stimulation of abscission is shown in table I. Earlier experiments, in which the effects of these gases on other physiological processes were studied, are included in this table to demonstrate the similarity between the various systems. There appeared to be a good correlation between data on abscission and growth inhibition of pea stem. The earlier work with tobacco growth inhibition (27) and petiole epinasty (14) did not correlate as well. Contamination of the test gases with ethylene was







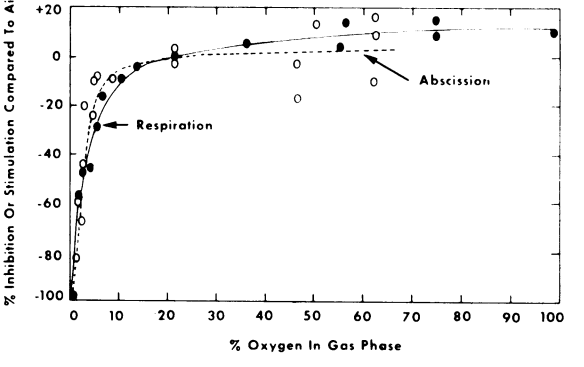


FIG. 1. (top, left) Effect of unsaturated aliphatic gases on abscission of bean petiole explants.
FIG. 2. (top, right) Effect of carbon dioxide on abscission of explants from 4 different species.
FIG. 3. (bottom, left) Reversal of carbon dioxide inhibition of abscission by ethylene.
FIG. 4. (bottom, right) Effect of oxygen on respiration and abscission of bean petiole explants.

Table I. *Relative Biological Activity of Ethylene and Other Unsaturated Gases*

Compound	Abscission	Inhibition of growth		
		Pea stem (9)	Tobacco (27)	Epinasty (14)
Ethylene	1	1	1	1
Propene	60	100	100	500
Carbon monoxide	1250	2700	1670	5000
Acetylene	1250	2800	100	500
Vinyl fluoride	2500	4300	...	...
1-Butene	100,000+	270,000	2000	500,000
1,3-Butadiene	100,000+	5,000,000	...	...

not reported, and may account for the greater activity of acetylene and 1-butene.

Carbon Dioxide. Figure 2 presents data showing that the abscission of explants from 4 different species was blocked by carbon dioxide. As shown in figure 3, the effect of carbon dioxide could be reversed by simultaneous addition of ethylene. Higher concentrations of ethylene were required to overcome higher concentrations of carbon dioxide.

Oxygen. The rate of oxygen uptake as a function of oxygen concentration is shown in figure 4. Compared to air, half-maximum inhibition of respiration occurred at 0.25 % oxygen, while respiration was maximal above 60 % oxygen. The commercial oxygen used in these experiments was contaminated with 0.2 ppm ethylene. However, we found in these studies that up to 85 ppm ethylene had no significant effect on respiration of either freshly harvested or aged explants. The rate of abscission as a function of oxygen concentration was plotted in the same figure to facilitate comparison. The oxygen used in these experiments contained 0.025 ppm ethylene. Half-maximum amounts of ethylene (0.1 ppm) were added to the gas phase after adjustment of the oxygen levels in order to determine whether oxygen potentiated or otherwise enhanced the effectiveness of ethylene. Within the 4 hours allotted to these experiments, oxygen had no observable effect on ethylene production from the explants. The data shown in figure 4 indicate that inhibition of abscission at low oxygen concentrations was the same or less than the inhibition of respiration. Unlike respiration, increasing oxygen concentrations above 21 % did not increase abscission.

Discussion

Burg and Burg (9) pointed out that the attachment site of ethylene could be characterized by its relative sensitivity to various unsaturated aliphatic compounds, the competitive action of carbon dioxide, and a requirement for molecular oxygen. They described the relative sensitivity of pea stem growth and fruit ripening to ethylene analogues and found that their results agreed with other investigations of similar compounds on other ethylene-sensitive systems. Table I presents similar data using abscission of bean petiole explants as the ethylene sensitive system. Based on the use of ethylene analogues, we conclude that the ethylene attachment site is similar for abscission, growth inhibition, fruit ripening, and epinasty.

Carbon dioxide blocks the ability of ethylene to inhibit root (13) and stem (9) growth, fruit ripening (17), celery blanching (19), flower wilting (24) and abscission (3, 26). Toole *et al.* (25) reported an exception in that the germination of peanut seeds was promoted by both ethylene and carbon dioxide.

By adapting Michaelis-Menton kinetics and Lineweaver-Burk plots to the carbon dioxide reversal of ethylene inhibition of growth, Burg and Burg (9) and Chadwick and Burg (13) demonstrated that carbon dioxide acted as a competitive inhibitor of ethylene. It was not possible to use these techniques in our abscission experiments because abscission could not be expressed as a rate phenomenon. However, the data in figure 3 suggest that the same interpretation can be made for the action of carbon dioxide on abscission. As the level of carbon dioxide surrounding the explants increased, more ethylene was required to promote abscission. Additional support for the hypothesis that carbon dioxide was a competitive inhibitor comes from earlier experiments of Yamaguchi (26) and Biggs and Leopold (6). Yamaguchi observed that the inhibition of abscission by carbon dioxide could be overcome by ethylene, and Biggs and Leopold observed that removing carbon dioxide from the atmosphere accelerated abscission.

There are a number of reports on the effects of oxygen on abscission (11, 12, 22, 23, 26). Carns *et al.* (12) found that maximum rates of abscission occurred when the gas phase contained 50 % oxygen. However, ethylene is a common contaminant of commercial oxygen in our case 0.2 ppm, and it is possible that the increased abscission above 20 % oxygen was due to increasing amounts of ethylene present as a contaminant. Using commercial oxygen, results similar to those reported by Carns *et al.* (12) were obtained. Yamaguchi (26) found that the maximum rate of abscission occurred at oxygen concentrations in excess of 10 % when ethylene was added to the gas phase. Other workers (22, 23) also observed an inhibition of abscission at low oxygen tensions.

In the experiments reported here, 0.1 ppm ethylene was added so as to cause half-maximum stimu-

lation of abscission. If oxygen played a potentiating or enhancing role in the action of ethylene, it should have been possible to observe a stimulation of abscission at oxygen levels above saturated respiration, or a greater inhibition of abscission than respiration at levels below saturation. However, we failed to observe either of these effects. Figure 4 indicated that the rate of abscission levels out sooner than respiration at high oxygen concentrations, and respiration is inhibited more strongly than abscission at lower levels of oxygen.

There is a lack of agreement on the role of oxygen in ethylene mediated phenomenon based on the results presented here and those advanced by Burg and Burg (9). Our results indicate that low levels of oxygen retarded abscission only because respiration was inhibited. Carns (11) has shown that abscission of bean petiole explants was blocked when they were treated with inhibitors of respiration.

Burg and Burg (9) suggested that zinc may be a part of the ethylene binding site because zinc-deficient tomato plants failed to respond to the gas. The role of zinc in abscission has not been fully established, but Brown and Wilson (6) reported that cotton plants, low in zinc, lost their leaves sooner than controls. Carns (11) found that as little as 6.5×10^{-5} M $ZnCl_2$ inhibited abscission of bean explants.

In general, it appears that the ethylene attachment site can be characterized by the relative sensitivity of ethylene to ethylene analogues and by the fact that carbon dioxide usually acts as a competitive inhibitor. It appears that the ethylene attachment site serves as a unifying feature in the action of this gaseous hormone in a number of different physiological processes. It will be interesting to learn how 1 particular combination of ethylene with the cell gives rise to a large number of different plant responses.

Acknowledgments

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