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Short Communication

A Method for Determining the Concentration of Ethylene in the Gas Phase of Vegetative Plant Tissues[1]

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Direct measurements of the internal concentration of ethylene in plant tissues have been limited almost entirely to fruit tissues (1, 11). In the case of many fleshy fruits, such measurements are routinely made by simply using a syringe to withdraw a sample of gas from the cavity of the fruit and then to inject it directly into a gas chromatograph for ethylene analysis (3, 8). In the case of fruits or fruit tissues where gas samples cannot be readily removed with a syringe, other methods have been used to a limited extent. Burg and Burg (6) collected gas samples from apple tissue slices by compressing the tissue in a syringe. Lyons *et al.* (8) used a vacuum technique to extract internal gases from young cantaloupe fruits. The data obtained by these methods have demonstrated that physiologically active concentrations of ethylene do accumulate in many fruit tissues prior to ripening (1, 3, 5, 8, 11).

In contrast to fruit tissues, the majority of the data on concentrations of ethylene in vegetative tissues have been obtained by indirect methods (2). In fact, direct measurements of the internal ethylene concentration in vegetative tissues have been made only in the case of bean hook hypocotyl tissue (7). This lack of information is due to the difficulties encountered in extracting gas samples from vegetative tissues.

In the course of our investigation into the possible physiological roles of ethylene in leaf abscission, we thought it essential to know on the basis of direct measurements whether or not physiologically active concentrations of ethylene occur within the leaf prior to abscission. Since no generally accepted method was available for making such measurements, we have investigated the possibility of using a vacuum to extract internal gases followed by gas chromatography for their identification. This report describes the apparatus and methods of extracting gases from within the tissue and a means for determining the validity of the method.

The apparatus for extracting the intercellular gases from plant tissues is illustrated in Figure 1. It consists of an evacuation chamber and a collection flask. The evacuation chamber is connected to a Matheson no. 49 vacuum regulator which in turn is connected to a vacuum source. The evacuation chamber is a 25-cm glass desiccator while the collection flask consists of an inverted 2-liter beaker the bottom of which has been drawn out into the shape of a funnel. A small hole in the tip of the funnel is fitted with a 6-mm rubber vaccine cap. The size of the collection flask and evacuation chamber depends largely upon the type of tissue to be examined and the amount required to obtain enough gas for analysis.

In principle the method of extracting the gases from the tissue is similar to the vacuum extraction method described by Magness (9). A vacuum is applied to a tissue completely immersed in a liquid causing the gases within the tissue to expand, escape, and collect over the liquid.

Routinely, the intercellular gases are extracted as follows. The evacuation chamber is filled almost to the top with a saturated solution of $(NH_4)_2SO_4$. A salt solution is used in preference to H_2O since the presence of the salt significantly reduces the solubility of ethylene (Fig. 2). The collection flask is completely immersed in the salt solution and air bubbles adhering to the inner surface of the flask and rubber vaccine cap are removed. The tissue to be extracted is immersed in a 0.01% solution of a surfactant (*e.g.*, Tween 20) to reduce adhesion of air bubbles and immediately introduced into the bottom of the collection flask by slightly tilting it. The tissue is gently rotated with the fingers to free adhering air bubbles which are quickly removed with a syringe. In order to prevent the collection flask from tipping over when the gases from the tissue collect at the top of the flask, the level of the extraction liquid is lowered to approximately 1 inch above the bottom edge of the flask. The evacuation chamber is quickly sealed, and a constant vacuum of 100 mm of Hg is applied for 2 min. This causes the gases within the tissue to expand rapidly and escape from the tissue, after which they collect at the top of the collection flask. At the end of the 2-min evacuation period, the chamber is opened, and a sample of the gas is removed with a syringe and injected directly into a gas chromatograph for analysis. Details of the chromatographic procedure were as previously described (10). The total time required for the extraction operation is usually less than 6 min.

In order to verify the accuracy of the vacuum extraction method, we used it to determine ethylene levels of predictable magnitude, specifically those in tissues treated with known levels of ethylene. The internal ethylene concentration of a fruit treated with ethylene is increased by the level of gas applied in accordance with Fick's law (2, 4). Thus, if the vacuum extraction method gives a reasonably accurate measurement of the internal ethylene concentration of a tissue, the internal ethylene concentration of a treated tissue should be increased by an amount equal to the amount of ethylene applied. The major potential error of the vacuum extraction method is that ethylene in the dissolved or bound state may be released when the vacuum is applied to the tissue. This possibility was tested by determining ethylene levels in tissues treated with ethylene. If a significant amount of ethylene is released into the gas phase when vacuum is applied to a tissue, then ethylene applied exogenously to the tissue before extraction should cause the internal concentration, as determined by vacuum extraction, to be greater than the predicted value. This conclusion assumes that the release of ethylene into the gas phase would be

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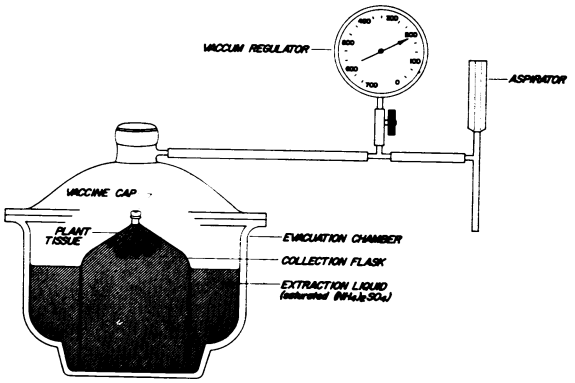

FIG. 1. Apparatus for extracting intercellular gases from plant tissues without contamination with air.

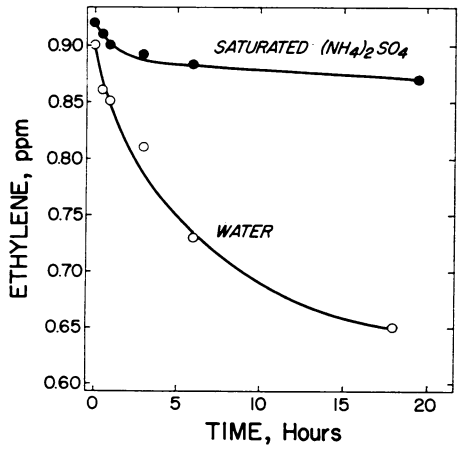

FIG. 2. Time course of the decrease in the ethylene concentration of a gas sample in contact with H_2O or a saturated $(NH_4)_2SO_4$ solution under conditions similar to those used in the vacuum extraction method. Thirty cubic centimeters of air containing either 0.90 or 0.92 μl/liter of ethylene were released as small bubbles under the bottom edge of five collection flasks containing either H_2O or saturated $(NH_4)_2SO_4$. At various times thereafter samples were removed and analyzed for ethylene by gas chromatography. Data are averages from two experiments.

greater for treated plants than those not treated. The above procedure will not allow absolute verification of the method. However, one can at least conclude that if treatment of tissue with ethylene does not result in a large increase in internal levels (observed by vacuum extraction) over calculated levels, then the levels (observed by vacuum extraction) in nontreated tissue should be elevated over the true level to no greater degree.

Initial experiments in which plants were treated with ethylene in sealed containers for 24 hr, quickly transferred to the vacuum extraction apparatus, and then extracted failed to give the predicted internal ethylene concentration. Tissues treated with 100 μl/liter of ethylene had internal ethylene concentrations of less than 1 μl/liter. This anomaly suggested that ethylene was being lost rapidly during the transfer of the tissue from the treatment chamber to the collection flask. The apparatus shown in Figure 3 was used to avoid this apparent error.

Intact plants were grown in hydroponics in a growth room under the following conditions: light intensity, 1800 ft-c; temperature, 30 C day, 25 C night; relative humidity, 70% constant; day length, 13 hr. Selected plants were sprayed to the point of run off with a 0.01% solution of Tween 20. The plants were covered with a 4-liter collection flask and then placed inside a 13-liter evacuation chamber and treated with ethylene. A similar lot of plants handled in an identical manner but receiving only room air served as the blank for determining the initial or background concentration of ethylene in the tissue. At the end of the ethylene treatment period 1-cc samples removed from the top and bottom of the bell jar were analyzed for ethylene to determine the treatment concentration just prior to extraction. Next, the water in the chromatography jar was removed by applying a vacuum through port A. Saturated $(NH_4)_2SO_4$ was then allowed to enter slowly into the bell jar through port B. As soon as a positive pressure developed within the bell jar, ports C and D were opened to allow the displaced air to escape. Intermittant, brief applications of vacuum to port D maintained similar rates of filling inside and outside of the collection flask. When the collection flask was completely filled with liquid, port B was closed and the rubber stopper in the top of the bell jar was removed. Any air not displaced from the collection flask by the liquid was removed with a syringe. In the case of the treated plants, this operation sealed the intercellular gases within the plant without exposing them to an air concentration of ethylene below the treatment concentration. After replacing the rubber stopper, the level of the liquid outside the collection flask was lowered and the internal gases were extracted and analyzed for ethylene as described above.

The results with this technique, in contrast to our initial results, were in close agreement with the predicted internal ethylene concentrations based on the sum of the blank and the known ethylene treatment concentrations. The internal ethylene concentration of three different plant species treated with various ethylene concentrations seldom varied more than 10% from the predicted value (Table I). These results suggested that in the case of the vegetative tissues tested the vacuum extraction method gives a reasonably accurate measurement of the internal ethylene concentration of the tissue. Prior to application of the vacuum extraction method to other species, one should verify experimentally that the internal ethylene concentration in the treated tissue in question equals the predicted value. This procedure will verify that the method is measuring gas phase ethylene.

Both the amount of vacuum applied and the length of time tissue is subjected to vacuum appear critical. We have observed that lowering the vacuum below 100 mm Hg, which also increases the time that tissue is exposed to vacuum, increased the values obtained for native ethylene in cotton tissue and fruit. Whether the reduced vacuum induces a wound production of ethylene or results in a release of a significant amount of bound or dissolved ethylene is not clear. It does appear advisable, however, to use the

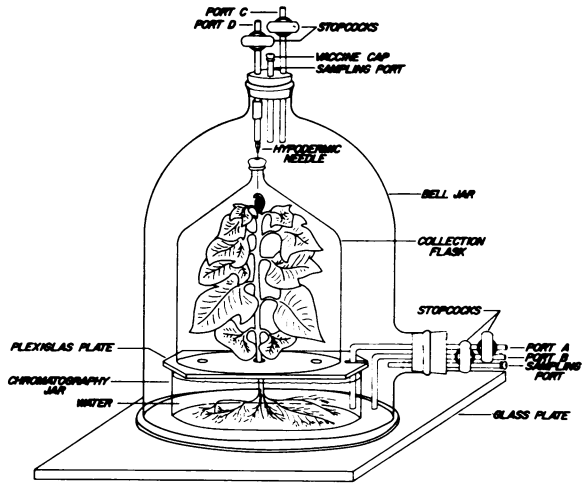

FIG. 3. Apparatus for treating plant tissues with ethylene and extracting intercellular gases from the tissue.

Table I. *Effect of Ethylene Treatment on the Subsequent Internal Ethylene Concentrations of Various Plant Species as Determined by the Vacuum Extraction Method*

Species	Age	Treatment[1] Concn	Internal Ethylene Nontreated Plants	Predicted Internal Concn of Treated Plants	Observed Internal Concn of Treated Plants	$\frac{\text{Observed Concn}}{\text{Predicted Concn}} \times 100$
				μl/liter		
Cotton, *gossypium hirsutum* L., cv. Stoneville 213	22 days	11.10	0.53[2]	11.63	11.00	94.6
	24 days	11.25	0.53[2]	11.78	12.80	108.7
	27 days	7.30	0.53[2]	7.83	8.30	106.0
	29 days	4.22	0.53[2]	4.75	5.00	105.3
	28 days	1.17	0.53[2]	1.70	1.80	105.9
	24 days	1.09	0.53[2]	1.62	1.80	111.1
	19 days	0.87	0.53[2]	1.40	1.67	119.3
	Average					107.3
Beans, *Phaseolus vulgaris*, L., cv. Red Kidney	18 days	0.98	0.61	1.59	1.63	102.5
	19 days	1.10	0.47	1.57	1.77	112.7
	16 days	0.85	0.50	1.35	1.40	103.7
	18 days	0.29	0.39	0.68	0.67	98.5
	18 days	1.41	0.48	1.89	1.98	104.8
	20 days	3.69	0.52	4.21	4.41	104.8
	21 days	6.20	0.74	6.94	7.60	109.5
	Average					105.2
Coleus blumei Benth., Princeton clone	7 months[3]	1.10	0.85	1.95	2.10	107.7
	7 months	1.08	0.33	1.41	1.80	127.7
	3 months	1.17	0.58	1.75	1.80	102.9
	3 months	2.58	0.59	3.17	3.23	101.9
	3 months	0.95	0.50	1.45	1.66	114.5
	Average					110.9

[1] Plants exposed to ethylene for 24 hr in most experiments and 23 or 25 hr in a few.

[2] Average of three separate blank determinations.

[3] Older coleus had been cut back twice; younger coleus was excised stems taken from vegetative plants at the age indicated.

minimal vacuum necessary to release enough gas for analysis and not to lower the vacuum below 100 mm of Hg.

We have found that gas samples removed from fruits with a syringe generally contain less ethylene than samples obtained from the same fruit by the vacuum extraction method. For example, Winesap apples and cantaloupes averaged 20 and 38% higher ethylene levels by the vacuum extraction method than by direct internal samples (data averages of 9 and 11 fruits, respectively, obtained from a local market). Lyons *et al.* (8) have also observed that vacuum extraction of young cantaloupe fruits gave ethylene values higher than anticipated. These results suggest that in the case of fruit tissues the applied vacuum may release a significant amount of bound or dissolved ethylene. Alternatively, some other factor may explain the failure of the method to determine internal ethylene levels accurately in fruits. Studies are being continued to resolve this question. It should be pointed out that the average ethylene levels, by direct internal sampling, were 165 and 686 μl/liter in cantaloupes and apples, respectively. Our observed values ranged from 30 to 1100 μl/liter. These values are orders of magnitude greater than those we observed in vegetative tissue or used to treat plants (Table I). For this reason, the problem with fruit does not invalidate use of the method to determine natural ethylene levels in vegetative tissue. It could be that vegetative tissue containing 100 to 600 μl/liter of ethylene would give similar results.

The method appears most applicable for determining relative ethylene levels or differences between various tissues. The values for internal ethylene levels obtained are directly correlated with ethylene production rates (unpublished data), and their accuracy as absolute values remains to be completely verified.

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