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Plant Responses To Naturally Emanated Volatile Compounds From Ripe Fruits

WAYNE C. HALL

This work was undertaken in an attempt to demonstrate the effects of natural emanations from fleshy fruits upon various plant organs and to devise a procedure which would be suitable for use as an experiment in an elementary plant physiology course or as a demonstration in general botany.

The fact that plants produce volatile compounds as the result of normal metabolism has been known for some time. As early as 1908 Crocker and Knight answered the gardener's question, "What causes carnations to go to sleep", by showing that ethylene of escaped illuminating gas could induce permanent "sleep" in the carnations within twelve hours. Their work stimulated further study of the effects of ethylene upon plants. In 1913 and 1917 Harvey and Doubt, respectively, recorded epinastic responses of certain leafy plants when subjected to a low concentration of ethylene in air. Later other investigators also presented evidence that epinasty is due to natural plant emanations especially from fruits. Elmer (1932) noted that when potato tubers and apple or pear fruits were stored in the same bin or chamber sprouting of potatoes were considerably delayed, and that shoots initiated under these conditions developed abnormally. Botjes (1933) demonstrated epinasty of the tomato plant by enclosing it under a bell jar with a single apple while Huelin (1933) pointed out that effects similar to those produced by apple fruit could also be obtained with small amounts of ethylene gas. He suggested that ethylene was the effective component arising from apple fruits. Proof of the chemical nature of the gaseous products given off by apple fruits was obtained by Gane (1934). This worker collected the volatile products originating from sixty pounds of Permaine apples over a period of four weeks in bromine at -65°C. Fractional distillation yielded a compound which when heated with aniline and crystalized from dilute alcohol agreed with the melting point of N.N'-diphenyl ethylene diamine (M. P. 62.5°C.). In a series of papers Denny aand Miller (1935, 1936, 1938) have presented data showing that emanations, principally ethylene, arise from various plant tissues and they recorded additional plant responses due to this gas. Using young potato plants as an indicator of the epinastic response, Denny and Miller (1935) noted positive results in the case of the fruits of apple, pear, tomato, banana, cantaloupe, squash, egg plant, avocado, and loquat; partially ripe seeds of lima beans and peas, and various other reproductive and vegetative tissues of a wide range of species. Also these workers concluded that ethylene is the only gas effective in causing epinasty of tomato petioles at very low concentrations and that the magnitude of ethylene evolved in their experiments probably approached one part in twenty million by volume.

In light of the previous work on the effects of ethylene it was felt that ripe fruit would be a suitable source in studying the effects of natural emanations upon various plant organs. It also seemed feasible to devise a relatively simple qualitative method for identification of the nature of the volatile products arising from ripe fruits.

METHODS AND RESULTS

I. Experiments with Natural Emanations

In initial work the ripe fruits of red delicious apples, lemons, and tomatoes were employed as the source of the volatile emanations and the effects observed on various plant structures. The test material was placed under a bell jar with the fruit and the base of the jar sealed on a glass plate with vacuum wax. Controls, identical with the test material except for absence of the fruit were run in all cases (Fig. 1).

All plants were exposed to natural greenhouse lighting and in experiments that ran over two days the plants were watered as necessary to avoid the effect of water as a limiting factor. During this study the effects of emanations from ripe fruits upon various parts of the following plant species were investigated:

1. The foliage of Nicotiana tabaccum, var. Little Turkish, and Nicotiana Langsdorfii at two stages of development.
2. Flower closure and discoloration of carnations blossoms.
3. Discoloration and abscision of flowers in white single petunia, snapdragon (var. Bronze Scarlet), and hibiscus.
4. The sweetening of Irish cobbler tubers by hydrolysis of starch.
5. The foliage and blossoms of geranium.
6. Young tomato plants (var. Pan American).

Whenever possible entire potted plants were used and when this was not feasible a cut plant in an erlenmeyer flask filled with tap water was employed. After several preliminary trials it was found that apple fruits were most effective and use of other plant sources of ethylene was subsequently discontinued. The best results were obtained with firm ripe fruits, neither very green nor extremely ripe. Apples that had attained the soft, mealy stage were totally ineffective.

OBSERVATIONS

Tobacco. In the experiments with two varieties of tobacco enclosed in the same container at 18°C. with ripe fruits the following sequence of events was observed:

In 8 Hours—slight epinasty and marginal curling of the leaves (downward).

In 24 Hours—slight loss of green color in basal leaves, accentuated leaf curl, and slight wilting.

In 48 Hours—definite loss of green color in basal leaves, marked curling of upper leaves.

In 60 Hours—all but uppermost leaves deep lemon in color. Color loss originated at edges and tips of laminae and moved progressively inward towards mid-rib and petiole.

After 72 Hours—blackening and necrosis at the edge of laminae.

The control plants (Fig. 1) remained healthy over the same period

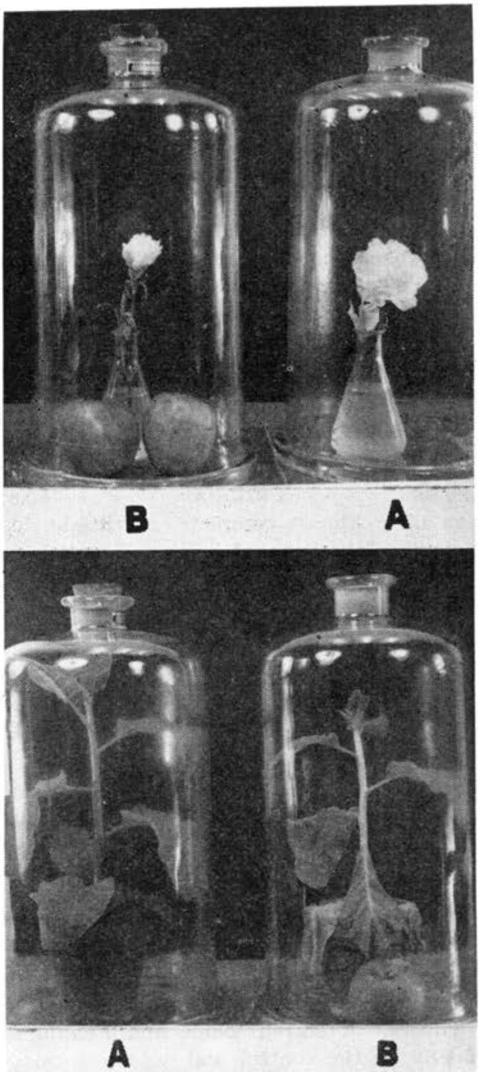

Fig. 1. Typical Responses of Young Tobacco Plants and Carnation Blossoms to Natural Emanations from Apple Fruits. A. Controls. B. Plants Exposed to Fruit Emanations.

of time, the leaves retained a normal green color, and no visible effects of enclosure under the bell jar were evident except that slight epinasty occurred but to a lesser degree than in the treated plants. It was also noted that apple fruits were the most effective in producing these symptoms and that young tobacco plants in the early rosette stage were more susceptible than older plants in the elongation stage of the two varieties tested. Growth was impaired and plants with flower buds seemed to exhibit some adverse effects in subsequent flowering even after removal from the bell jar. Upon being returned to the greenhouse after treatment, plants with affected leaves failed to recover. Lower leaves were dead and in time dried completely and abscised.

Cut Carnations. Flowers in bloom (Fig. 1) responded uniformly as follows in a number of experiments when subjected to fruit emanations:

In 8 Hours—no appreciable effects .

In 24 Hours—blossoms were about 50% closed, with slight discoloration of petals.

In 48 Hours—blossoms completely closed, discoloration and browning of petals, often infected with mold.

The control blossoms remained completely open throughout sometimes expanding even more fully than originally.

Uncut Flowers. Discoloration and abscision of petunia, snapdragon, and hibiscus flowers:

Time	HIBISCUS	PETUNIA	SNAPDRAGON
In 24 hours	Abscision of all blossoms, slight leaching of red color.	Almost complete abscision of blossoms.	Slight loss of bronze-scarlet color of petals.
In 48 hours	Experiment terminated.	Complete abscision of flowers, browning and yellowing of white flowers.	50% of blossoms abscised, flowers pale, whitish pink.
In 60 hours	Experiment terminated.	Lower leaves chlorotic.	Complete abscision and brown discoloration of flowers. Plant infected with mold.

In all cases the control plants remained healthy and no adverse effects were observed throughout except that the snapdragon plants became slightly infected with mold after prolonged exposure in the high humidity of the sealed bell jars.

Irish Cobbler Tubers. A starch iodide and Fehling test was performed periodically on the control and experimental tubers. At the end of the thirteenth day both gave a positive starch test and experimental tubers gave slight indications of a positive sugar test with Fehling's reagent. Therefore thin sections were made and a

Flückiger test performed. Glucose and fructose crystals were identified microscopically, their presence indicating definite starch hydrolysis in tubers exposed to apple emanations.

Geranium and Tomato Plants. In the case of geranium the control plant was healthy and green at the end of four days with the flowers still bright red and intact, whereas the experimental plant began to manifest responses to emanations at the end of two days; flowers abscised and the red color of corollas faded. the lower leaves became chlorotic and dried. In four days most flowers had become discolored and dried. Most flowers abscised and the plant had become badly chlorotic with complete necrosis of most of the lower and middle leaves. Though not determined specifically, there is the possibility that some of the effects noted were due in part at least to volatile amines since such emanations also occur naturally, especially from flowers. The effect of such emanations might be expected to become distinctly evident in plants in a closed container.

Tomato plants displayed the typical epinastic response with yellowing and defoliation occurring after prolonged exposure. The control plants did not show these effects.

II. Experiments with Ethylene Gas

Ethylene gas was generated by the dehydration of alcohol by placing thirty milliliters of 95% ethyl alcohol in a distilling flask and adding slowly eighty milliliters of concentrated sulfuric acid. When the reagents were thoroughly mixed the flask was heated to 170°C. and the ethylene fraction distilling over at this temperature was washed by bubbling through 3-Normal sodium hydroxide and the gas collected over water. Three insulated bell jars were then assembled (Fig. 2). One contained the test plant under the bell jar with ethylene gas; the second consisted of a test plant exposed to the emanations of two apples; the third was the control jar containing only the plant. The effects of these three variable upon plant materials consisting of potted healthy tobacco plants, var. Little Turkish in the elongation stage and cut carnation blossoms were noted.

The attempt to duplicate by means of ethylene gas some of the responses resulting from fruit emanations showed that the same results are obtained in elongating tobacco plants and carnation blossoms by either ethylene gas or by fruit emanations (Fig. 2). The effects of the free gas however are more severe which is probably attributed to the much higher concentrations of ethylene gas employed in contrast to the extremely low concentrations originating from fruit (free ethylene gas employed approached ten parts per thousand by volume).

III. Effect of Intact Ripe Tomato Fruits upon Various Organs of the Parent Plant

An assembly was devised (Fig. 3) whereby the top portion of a

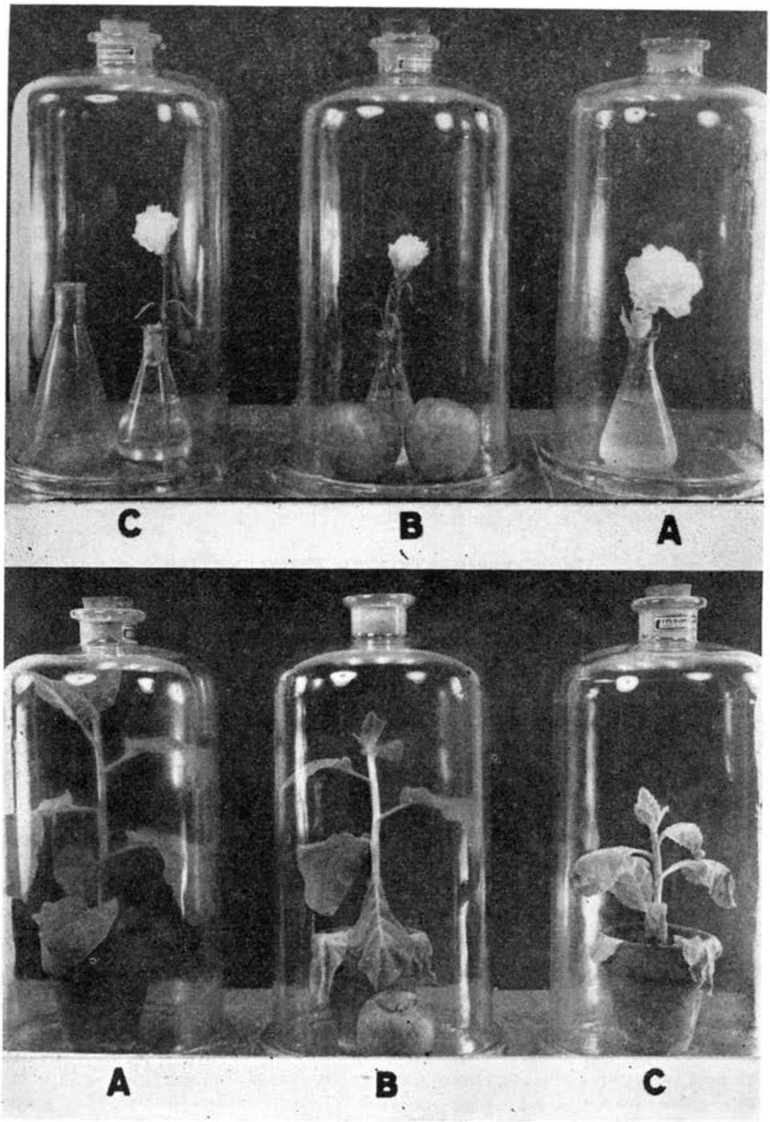

Fig. 2. Typical Responses of Young Tobacco Plants and Carnation Blossoms Showing Comparable Results Obtained by Both Free Ethylene Gas and Natural Fruit Emanations. A. Control. B. Plant Exposed to Fruit Emanations. C. Plant Exposed to Free Ethylene Gas.

large mature potted tomato plant with ripe fruits was enclosed in an insulated glass chamber and the remainder of the plant exposed to the normal atmosphere to determine if the effects observed by

Skok (1943) could be repeated. A second completely defruited plant was similarly treated except ethylene gas was substituted for the removed fruit. A defruited plant without fruits or free ethylene gas was also tested as a control.

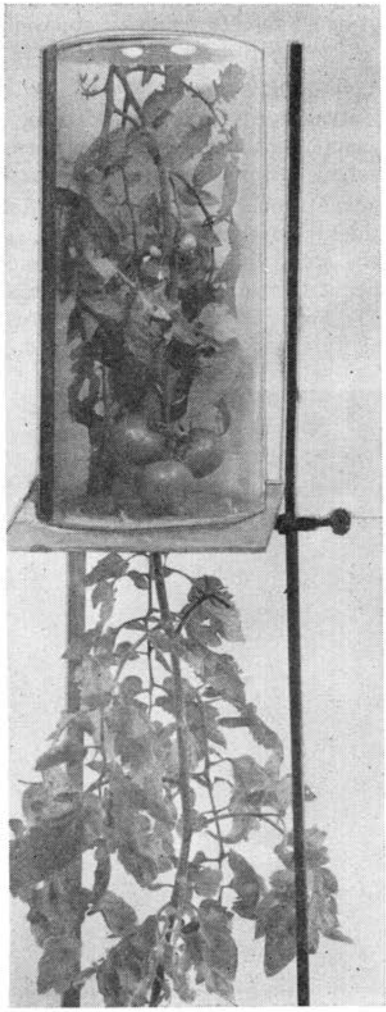

Fig. 3. Laboratory Assembly Employed to Demonstrate That Intact Ripe Fruits of Tomato Will Elicit the Same Responses as Free Ethylene Gas Upon the Parent Plant.

In an extensive study of the defoliation of tomato plants, both under laboratory and field conditions, Skok (1943) reached the conclusion that the major factor in defoliation and frequent low yield of fruit was due to the gaseous emanations, chiefly ethylene, from ripe fruits. In the present repetition of Skok's tests the following series of events were observed in two separate trials with the laboratory assembly described (fig. 3).

In 14 Hours—typical tomato epinasty apparent, some abscision of young flowers.

In 48 Hours—yellowing and chlorosis of the leaves.

In 72 Hours—intensified yellowing of leaves, petioles abscised, small root-like appendages formed on portion of stem enclosed by the glass chamber.

In 6 Days—adventitious roots, some over one inch long and thickly covered with root hairs, completely covered the stem under the chamber and terminated only about two inches from the apex of the stem (Fig. 5). Extreme defoliation, chlorosis, abscission of flowers and fruits had occurred.

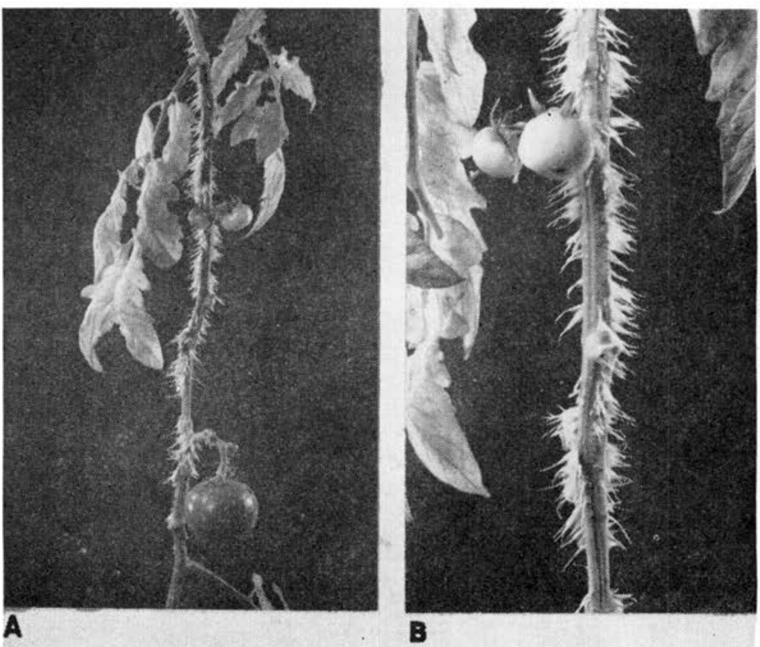

Fig. 5. The Production of Adventitious Roots Upon the Aerial Stem of Tomato as the Result of Exposure to Natural or Free Ethylene Gas. A. Photograph Showing Adventitious Roots on Portion of Aerial Stem Under Glass Chamber. Approximately One-fourth Life Size. B. Enlarged View Showing Root Hairs in More Detail. Approximately One-half Life Size.

The above data confirm and slightly extend Skok's results. As a check to determine if emanation of ethylene was the effective agent in initiating the above responses, a defruited plant was enclosed in the glass chamber and exposed to an ethylene atmosphere. after a beaker of anhydrous calcium chloride had been introduced into the chamber to absorb excessive moisture. The same characteristic symptoms reported above were observed, including root formation from the stem within sixty hours. Defruited control plants failed to give these effects and only a slight foliar epinasty was noted. Histological examination disclosed that the root-like structures arise endogenously, apparently from the cambial or xylem region of the stem.

IV. Identification of Emanations of Apple Fruits

Nine large delicious apples were placed under a sealed bell jar (Fig. 4), the atmosphere evacuated slowly by the use of an aspirator and collected by bubbling through the following solutions: a dilute aqueous solution of potassium permanganate (rose colored) at concentration of 0.0125%, a 1% bromine-water solution, a 1% solution of bromine in carbon tetrachloride, 0.05% Schiff's reagent (basic fuchsin decolorized with sulfurous acid).

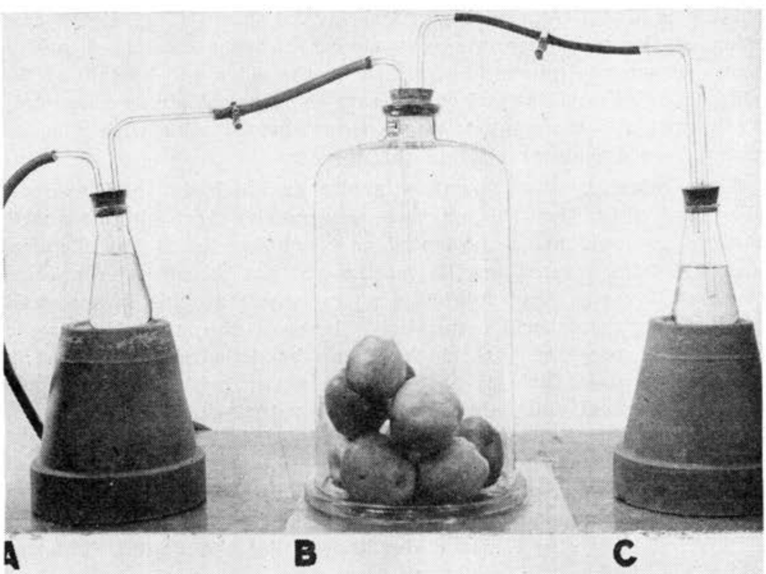

Fig. 4. Apparatus Used to Collect and Identify the Volatile Compounds Arising From Apple Fruits. A. Collecting Flask Containing Test Reagent and Tube Leading to Aspirator. B. Sealed Bell Jar with Apple Fruits. C. Scrubbing Flask with Tube to Atmosphere.

The atmosphere entering the bell jar was likewise passed through a scrubbing bottle containing the reagent under test in order to remove any contamination by unsaturated hydrocarbons that might be present in the laboratory air. The effects upon the four reagents were repeated several times to confirm results.

Investigations were undertaken to determine a simple qualitative test, preferably a colorimetric or indicator method for ethylene. It is known that a dilute solution of potassium permanganate is decolorized by compounds having an ethylenic or acetylenic linkage as the double bond is readily attacked by oxidizing agents, and this property is the basis of the Baeyer test for unsaturation in hydrocarbons (Fuson and Shriner, 1940). Logically, then, if we are dealing with an unsaturated hydrocarbon as the evidence seems to indicate, the first step is to test the reaction of the volatile emanations from apples upon aqueous permanganate. If ethylene is present then presumably the following reaction would occur:

$$3\ CH_2 = CH_2 + 2\ KMnO_4 \rightarrow 3\ \underset{\displaystyle OH}{\underset{|}{CH_2}} - \underset{\displaystyle OH}{\underset{|}{CH_2}} + 2\ MnO_2 + 2\ KOH$$

Ethylene would react with the rose colored permanganate to form ethylene glycol and manganous dioxide would form as a brown precipitate. After 14 hours of slow evacuation and collection of the volatile products from apples by the method shown (Fig. 4) the rose color of the permanganate was almost entirely dissipated and a heavy brown precipitate had formed on the sides and bottom of the collecting flask. At the end of 24 hours the rose color was completely discharged and the solution was a russet brown color with a heavy manganous dioxide precipitate throughout.

Since other common plant products as aldehydes and alcohols also give a positive Baeyer test, the positive test obtained with the apples could not be accepted as conclusive as to the chemical identity of the emanations. It was though that the effective product given off by ripe fruit might be acetaldehyde as this is a normal product of pyruvic acid respiration. Therefore the next step was to test for its presence. Acetaldehyde can be detected by the use of decolorized basic fuchsin, or Schiff's reagent, which is commonly used in chemical and bacteriological laboratories, and is known for its extreme sensitivity to this compound. After treatment with the gaseous emanations for periods up to ninety-six hours the reagent was still unchanged and it was concluded that an aldehyde did not occur in the emanations.

In the case of ethylene it is also known that bromine and chlorine add to the double bond readily to form ethylene bromide and in bromine water the color would be discharged with the formation of small, brownish colored, emulsified oil droplets. When a flask of bromine water was connected to the system, the color was discharged in five hours and the characteristic oil droplets (ethylene bromide) appeared in the liquid. A positive test for unsaturation however, is one in which the bromine color is discharged without the evolution of hydrogen bromide. Enols, phenols, aromatic amines, aldehydes, ketones, many esters, and numerous compounds containing active methylene groups react readily with bromine, but in all these reactions hydrogen bromide is formed (Fuson and Shriner, 1940). Bromine in carbon tetrachloride is an excellent test for the olefinic or acetylenic linkage as carbon tetrachloride is a good solvent for bromine but not for hydrogen bromide. Consequently the use of bromine-carbon tetrachloride solution would differentiate among the above mentioned compounds on the basis of whether or not hydrogen bromide is evolved, and whose presence can be detected by breathing across the top of the open vessel containing hydrogen bromide or by wet litmus paper. A positive test for hydrogen bromide will be apparent if a dense white cloud forms or in the change of color of the litmus paper. When a flask of bromine-carbon tetrachloride solution was connected to the system with the apples the color disappeared in sixty hours with the formation of oil droplets. When the breath was blown across the top of the collecting vessel a negative test for hydrogen bromide was obtained. However, when the scrubbing bottle of bromine-carbon tetrachloride solution was breathed upon a dense white cloud appeared.

The above tests give further proof that the volatile products from the apple fruits is not an alcohol, aldehyde, or carbonyl compound as alcohols decolorize permanganate solutions but not bromine solutions. Carbonyl compounds that decolonize bromine solutions give a negative Baeyer test and in the case of aldehydes, many decolorize permanganate solutions, but benzaldehyde and formaldehyde do not discharge the color of bromine water. Thus with plant materials the decoloration of aqueous permanganate accompanied by a precipitate of manganous dioxide and by formation of oily droplets of ethylene bromide in the case of bromine water is a reliable test for the presence of ethylene.

SUMMARY AND CONCLUSIONS

1. A variety of characteristic responses of plants to natural emanations of ripe, fleshy fruits are described; any or several of these can easily be adapted as an individual experiment for use in a physiology course or as a demonstration in general botany without elaborate equipment or great expense.
2. Several responses of plants to ethylene emanations are presented which to the author's knowledge have not previously appeared in the literature.
3. It was shown that intact ripe fruits of the tomato plant can under certain conditions produce typical ethylene effects in other parts of the parent plant.
4. The production of adventitious aerial roots at the apical portion of tomato stems both by natural fruit emanations and ethylene gas, suggests similarity of effect to growth substances as has been previously pointed out in the literature.

5. By combining the Baeyer test and the bromine-carbon tetrachloride tests for unsaturation, a fairly simple method for identifying the usual volatile products arising from ripening fruits is presented, well within the scope of beginning students.

6. Although these tests are not entirely conclusive, they do indicate that the compound is an unsaturated hydrocarbon, and of these, only ethylene, acetylene, or propylene could be effective at the extremely low concentrations arising from fruit emanations.

7. As suggested previously ethylene is 500 times more effective in inducing the characteristic plant responses than the other two gases, which would leave one to conclude that ethylene is the most effective component of natural emanations of fruits.

The writer wishes to express his sincere appreciation to Professor W. F. Loehwing for beneficial suggestions throughout the course of this problem and to Professor R. L. Shriner for helpful advice in the identification of the volatile fruit emanations.

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