Technical Papers

Nonspecificity of the Triple Response for Ethylene[1]

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The present note discusses the validity of the triple response of etiolated pea seedlings as a measure of gaseous ethylene production by living material. The triple response has been used frequently by physiologists as a measure of ethylene production by ripening fruit, by fungi, and by injured and healthy plant tissues. The evidence presented below suggests that when ethyl alcohol is also produced by living material, as with certain fungi, the triple response is unreliable as a specific detector of ethylene.

Living plant tissues may not only produce ethylene but also may be affected by it in very low concentration. Tomatoes respond epinastically to concentrations of ethylene in air as low as 0.1 ppm by volume and epinastic responses are shown by African marigold to concentrations of 0.001 ppm (*1*). Chemical methods for determining ethylene will usually not detect such low concentrations unless considerable quantities of material are available. For this reason bioassays for ethylene have been developed. The most commonly employed of these is the triple response of etiolated Alaska pea seedlings in which concentrations of ethylene as low as 0.05 ppm will induce a shortening of the epicotyl, a curvature of the apical plumule, and an increase in diameter of the epicotyl (*2, 3*). At very low concentrations only the epicotyl is shortened; at higher concentrations all three responses appear (*1*). The triple response is a quantitative test for ethylene, and has come to be widely accepted as a specific detector of ethylene arising from living material (*4*).

Certain materials that affect the growth of plants will not interfere with the triple response test. Gases such as acetylene, propylene, and carbon monoxide can be detected by the triple response in concentrations as low as 250, 1000, and 5000 ppm, respectively (*1*). These gases do not arise from living material, however. The known natural plant hormones other than ethylene are nonvolatile from plants and cannot interfere because of the manner in which the test is set up. However, volatile compounds other than ethylene arise from living material and the reliability of the triple response as a specific indicator for ethylene depends upon a demonstration that volatile compounds other than ethylene do not elicit the triple response in etiolated pea seedlings.

In recent studies dealing with the production of ethylene by the fungus *Fusarium oxysporum* f. *lycopersici*, it became necessary to measure the production of ethylene in the presence of ethyl alcohol. To determine whether or not ethyl alcohol affects the triple response, etiolated pea seedlings were exposed to alcohol vapor. Alaska peas[2] were soaked overnight in distilled water and were then transferred to filter paper sheets in Petri dishes, 75 seeds per dish. The dishes were watered with saturated $CaSO_4$ solution and were then placed in a dark cupboard at room temperature for 2 days. At the end of this time the 50 most uniform germinating seedlings were left in the dishes, the others were discarded. One Petri dish containing seedlings was placed in each of three empty desiccators (2.1 l capacity), containing 100 ml of water or alcohol solution, ranging in concentration from 0.1 to 1.0% by volume. Desiccators were then closed and placed in a dark cupboard for 3.5 days. At the end of this time the length and diameter of epicotyls and the angle of curvature of the growing point were measured. Epicotyl lengths were then converted to logarithms, whereas plumule angles and epicotyl diameters were not transformed before statistical analysis.

TABLE 1. Response of etiolated pea seedlings to ethyl alcohol vapor.

Concentration of alcohol (%)	Logarithm of epicotyl length (log mm)	Angle of plumule (deg)	Diameter of epicotyl (mm)
0.0	1.17	99	2.52
0.1	0.86	87	2.58
1.0	0.60	66	2.51
Least significant mean difference (P: 0.05)	0.06	12	0.10

Exposure to ethyl alcohol significantly reduced the length of pea epicotyls and the angle between the plumule and the epicotyl below (Table 1). These are the two responses commonly used by investigators to indicate a positive triple response. This result has been confirmed in repeated experiments.

Ethyl alcohol is frequently formed in biological material under circumstances where ethylene might be expected to occur. In the present study it was found that *Fusarium* produces both ethylene and ethyl alcohol in culture (*5*). Ripening fruit also produces both compounds simultaneously (*6*). Apparently where ethyl alcohol may be present, the triple response of etiolated peas cannot be used as a reliable indicator for the presence of ethylene.

When ethyl alcohol may be present, ethylene can be detected in a number of ways. Tomatoes respond epinastically in the presence of 0.1 ppm ethylene (*1*).

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[2] Supplied through the courtesy of Associated Seed Growers, New Haven, Conn.

In the present study, tomato cuttings exposed to alcohol vapor arising from solutions of alcohol ranging from 0.1 to 95% by volume did not respond epinastically. A safer method involves the quantitative manometric method in which ethylene is trapped by mercuric perchlorate and released again with hydrochloric acid (*7*). This method may not be sufficiently sensitive for measuring very small quantities of ethylene. In such a case, it has been found satisfactory to trap the ethylene in mercuric perchlorate solution and to release it again in a closed vessel of small volume in which tomato plants are confined (*5*). The epinastic response of these plants will then be specific for ethylene if the gas arose from biological materials or did not contain other unsaturated hydrocarbons. Alternatively, the triple response can be used as a quantitative measure of the ethylene released from perchlorate solution on addition of hydrochloric acid.

References

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Successful Transplantation of an Apparently Benign Neoplasm

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A spontaneous mammary pericanalicular fibroadenoma developed in a female albino rat of the Sprague-Dawley strain. Grossly the tumor was a hard, white, homogeneous, lobulated, encapsulated, palpable mass. Six months after complete removal, no recurrence was indicated.

As indicated in the treatise by Greenstein (*1*), some investigators consider benign tumors incapable of further growth by transplantation. Small pieces of the present tumor, 2 ml thick by 3–4 ml in diameter, were implanted subcutaneously along the milk line of albino female rats. Of the transplants, 4 of 4 in the first, 5 of 6 in the second, 6 of 8 in the third, and 3 of 4 in the fourth generation continued to grow into large tumors. Increase in the size of transplant was not evident for 4–7 wk after transplantation. If no increase was evident after 8 wk, no growth occurred later. Histology of the tumors remained the same in each generation.

Transplantations into 4 male rats and 4 female mice (Webster albino) have been unsuccessful. Transplantation by injection of macerated suspensions of the tumor have so far failed. The transplantations are being continued. Any significant change in the appearance or behavior of this tumor will be reported.

[1] Adelaide Skinner Fellow in Cancer Research.

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Circular Paper Chromatography II. Isatin as a Color Reagent for Amino Acids

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In a previous publication (*1*), studies were presented of the physical factors that may influence R_f values for the circular chromatographic method (*2, 3*). Among the advantages of this method is the possibility of cutting the chromatogram into a number of segments and using various color producing reagents for each segment. Thus, it is possible to identify an individual amino acid in a band containing several other amino acids by means of *specific* color reactions. A number of such specific reagents which give colors with one or several amino acids have been mentioned in the literature (*4, 5*). This is in contradistinction to the use of ninhydrin (*6–8*) as the coloring reagent "par excellence" which gives purple or blue colors with virtually all the amino acids found in protein hydrolyzates. Exceptions to this color reaction with ninhydrin are the imino acids, proline and hydroxyproline, which give faint yellow colors. It was for this reason that Acher, Fromageot, and Jutisz (*9*) first proposed the use of isatin in *n*-butanol-acetic acid solution as a highly specific reagent which gives intense blue-green colors for proline and hydroxyproline on paper chromatograms. Their use of this reagent is based upon the experimental studies of Grassmann and Arnim (*10, 11*) on the reaction of isatin with pyrrole ring compounds to give colored products.

The chemistry of isatin is discussed in the textbook of Morton (*12*) and in a review paper by Sumpter (*13*). These sources point out that isatin reacts with both imines and amines to form products which in many cases are colored. However, only sporadic references were found to the reaction of isatin with amino acids in general (*14, 15*). Although isatin is now generally used as a specific reagent for the identification of proline and hydroxyproline on paper chromato-

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