THE PHYSIOLOGY OF ETHYLENE FORMATION IN APPLES

By STANLEY P. BURG AND KENNETH V. THIMANN

DEPARTMENT OF BIOLOGY, HARVARD UNIVERSITY

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Introduction.—The toxic action of illuminating gas on plants has been known for nearly a century, and the fact that ethylene is the effective constituent was proved in 1901. The effects of ethylene include inhibition of growth, loss of geotropic sensitivity, onset of epinastic curvatures, acceleration of respiration, initiation of rooting, modification of pigments both in leaves and in fruits, and hastening of fruit maturation. The discovery that ethylene can itself be produced by ripening fruits gives even greater interest to these phenomena, since it appears that the gas can in some circumstances function as an endogenous growth-controlling factor, or in fruits as a “ripening hormone.” However, although there have been numerous studies on the action of ethylene, little is known about its formation. In large part this is due to the fact that it is produced in excessively minute quantities—measured in microliters per hour per kilogram of tissue—and existing methods are inadequate to follow its production with the precision and speed needed for physiological studies.

The advent of modern methods of gas chromatography has made possible a new approach to the study of ethylene formation in biological materials. In the experiments of which the present paper is a preliminary report, this approach has led to the development of a method about 1,000 times more sensitive than the assays which have been employed in the past. Some applications of this methodology to the problem of the biogenesis of ethylene will be described.

Method and Materials.—In brief, the method consists of injecting the gas sample into the end of an adsorption column consisting of 18 in. of Tygon tubing packed with aluminum oxide. The column is developed with helium, and the issuing gases detected and measured by a high sensitivity katharometer. Several features adopted in the construction of the katharometer combine to produce very nearly the theoretical limit of sensitivity which is obtainable by the thermal conductivity principle. The inherent noise level of the instrument was minimized by using filaments which had been spot welded to the lead-in wires; the filaments of the katharometer were run at the temperature (80° C) which gave the highest signal to noise ratio; the output signal was magnified with an amplifier which had a lower noise level than the katharometer when the latter was run at its optimal wire temperature; the signal produced for a given change in thermal conductivity was maximized by using very fine, high resistance coiled filaments suspended in long, narrow channels. In this way a combination of high sensitivity and low noise level could be achieved which allowed the detection of quantities of ethylene below $5 \times 10^{-3}$ $\mu$L, while the chromatographic column permitted the use of total gas volumes as large as 2 ml. By comparison, the manometric method generally used, even if modified by reducing the volume of absorbent to a minimum, cannot very well detect less than 10 $\mu$L of ethylene.

Figure 1 illustrates results obtained with the procedure outlined above, demonstrating its consistency, sensitivity, and ability to separate closely related com-

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pounds. For any one constituent, the peak height was found to be linearly related to the total volume of gas present throughout the range $10^{-2}$ to $10^8 \mu L$. The relation between peak height and quantity was determined for each gas by empirical calibration. Samples containing apple emanations characteristically separated

![Diagram](image)

**Fig. 1.**—Performance curves. (Each record reads from right to left.)

*Top:* Three successive gas chromatograms of approximately 0.5 ml samples of air which were withdrawn from a Baldwin apple with a syringe. At the point marked $S$, the sensitivity of the analyzer was increased $1,050 \times$. Vertical lines on the graph are spaced at 1-min intervals. Values for the percentage ethylene in the internal atmosphere calculated from the three peaks are 0.163, 0.160, and 0.163, respectively.

*Lower left:* A gas chromatogram of 0.5 ml of air containing $6.8 \times 10^{-2} \mu L$ of ethylene, i.e., about 13 ppm. The sensitivity was increased 58,000$ \times$ at the point marked $S$, and thereafter the full scale corresponds to $1.0 \mu V$. Vertical lines on the graph are spaced at 1-min intervals. A correction must be applied for the air “tail” preceding the sample reading, and for a slow battery drift which occurs at very high sensitivities.

*Lower right:* A fractionation of 1.5 ml of natural gas to which 0.25 ml of ethylene has been added. The peaks are (a) methane, (b) ethane, (c) ethylene, (d) propane, (e) isobutane, and (f) $n$-butane. The sensitivity was increased 33.2$ \times$ between $a$ and $b$, reduced 7.3$ \times$ between $b$ and $c$, increased 55.5$ \times$ between $c$ and $d$, and increased 4,000$ \times$ between $d$ and $e$. Vertical lines on this graph are spaced at 4-min intervals.
into two fractions on the aluminum oxide column: a large first band containing oxygen, nitrogen, carbon dioxide, and perhaps trace amounts of other volatiles, and a small second band containing only ethylene.

McIntosh apples, purchased at the local market and stored in polyethylene containers at 4°C, were used in all experiments. When the apples were transferred from cold storage to room temperature, they consistently showed a maximum rate of ethylene production after 10 hr and were used at this time. About a dozen plugs were removed from a single apple with a No. 5 cork borer; these were cut to 3.8 cm length, rinsed in tap water, dried, and weighed (ca 2.7 g each). The sections were then inserted in 5 ml hypodermic syringes, the bore of these syringes being just sufficient to accommodate the tissue. When the plunger of a syringe was set at 4.2 ml, less than 2 ml of air was present within the tissue and free air space. The actual air content was calculated from the facts that apple tissue has a density of 0.8, and a free air space of 33 per cent. Needles were attached and sealed with rubber stoppers; the tissue was then incubated for 60 min, after which time the stoppers were removed and the contents of each syringe pressed out. Apple tissue is soft enough to pass through the needle, and the entire gaseous contents were collected over water (the solubility of ethylene in water is so low that the concentration of the gas is essentially unchanged by this procedure). A gas sample was then drawn up in a 2-ml syringe and chromatographed. The rate of ethylene production was calculated from the total air and ethylene recorded in the analysis, and the initial air volume in the syringe, weight of tissue, and total time of incubation. Preliminary experiments showed that apple plugs extracted from various parts of the fruit have the same rates of ethylene production per gram within ±5 per cent, and this rate is the same as that of the whole fruit from which the sections were cut. The production rate of the whole apple was determined by a somewhat different procedure, which need not be detailed here.

Syringes containing apple tissue were exposed to various gas mixtures by placing them tip downward in a desiccator, evacuating the system, and running a known gas mixture into the desiccator. Water was then admitted through a separatory funnel fixed in the top of the desiccator, and the excess-pressure which developed was relieved through an overflow. When the tips of the syringes were covered with water, the desiccator lid was removed, and the syringe needles were added and stoppered under water.

Respiration rates of apple plugs were determined in constant pressure respirometers, a differential system with and without alkali being used for carbon dioxide measurements.

Counting of tritium-labeled ethylene was carried out in the gas phase after extensive purification by condensation and redistillation.

Results.—(1) Effect of evacuation: Since apples may contain large amounts of ethylene in their intercellular spaces, measurements of the rate of production can only be made after this gas has been removed; otherwise it might represent a considerable portion of the ethylene present after a short collection time. An experiment was therefore devised to ascertain whether evacuation had any effect on the subsequent rate of ethylene production. Total ethylene content was determined on unevacuated plugs after 30 and 90 min, and the production rate was calculated by subtracting the 30-min from the 90-min total. A second set of syringes, con-
TABLE 1

<table>
<thead>
<tr>
<th>Time, Min</th>
<th>Unevacuated Ethylene, μL/Kg</th>
<th>Evacuated Ethylene, μL/Kg</th>
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<tbody>
<tr>
<td>30</td>
<td>159.0</td>
<td>...</td>
</tr>
<tr>
<td>60</td>
<td>...</td>
<td>133.0</td>
</tr>
<tr>
<td>90</td>
<td>288.8</td>
<td>...</td>
</tr>
<tr>
<td>120</td>
<td>...</td>
<td>269.6</td>
</tr>
</tbody>
</table>

Rate: μL/Kg/hr

C₂H₄ initially present (μL/Kg) calculated

<table>
<thead>
<tr>
<th></th>
<th>129.8*</th>
<th>134.8 av.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>94.1</td>
<td>0</td>
</tr>
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* Ninety-minute total minus 30-min total.

taining duplicate samples from the same apple, was evacuated in a desiccator, and production of ethylene was determined after 60 and 120 min. The data in Table 1 show that the evacuated tissue maintained a constant rate of ethylene production of about 135 μL/Kg/hr for the 2-hr period, whereas the unevacuated tissue—although it initially contained about 94 μL/Kg of ethylene—produced 130 μL/Kg in a 1-hr period (between 30 and 90 min). Evacuation, therefore, has no effect on the subsequent rate of ethylene production. This fact also strongly suggests that none of the precursors of ethylene is volatile. In all further experiments the tissue was evacuated just before the syringes were sealed.

(2) Temperature dependence: The influence of temperature was investigated by incubating syringes containing tissue from a single apple in various temperature-controlled water baths. Figure 2 summarizes the results obtained. Over a 2-hr period, the optimal temperature was found to be 32°C, and the Q₁₀ between 10 and 25°C was 2.8. Over the same temperature range, the Q₁₀ values for oxygen consumption and carbon dioxide production were found to be 2.75 and 2.5 respectively; (the carbon dioxide production of the tissue declined during the 2-hr period.)
period, so the $Q_{10}$ for this process is not exact). At 40°C and at higher temperatures, ethylene production is severely inhibited, and tissue held at 40°C for 1 hr produces little ethylene during a subsequent hour at a lower temperature (see Fig. 2). However, the temperature inhibition is not irreversible, and after 5 hr at room temperature about 50 per cent of the temperature-induced inhibition usually disappears. Hansen11 observed a similar recovery of ethylene production after pears had been heat treated.

(3) Effects of nitrogen, oxygen, and carbon dioxide: Several workers have reported that ethylene production ceases under anaerobic conditions2, 11, 12. The effect of reduced oxygen partial pressure on the ethylene production and oxygen consumption of apple tissue plugs is shown in Figure 3. The points at the lower oxygen tensions are subject to some uncertainty because of the difficulty of making precise correction for the amount of oxygen consumed during the 1 hr experimental period. However, the data show clearly that the effect of oxygen tension on ethylene production is either identical with, or similar to, its effect on respiration. This suggests either that both processes donate electrons to the same terminal oxidase, or else that the production of ethylene is dependent upon aerobic respiration for energy or for substrate. Since the apple shows a strong Pasteur effect, it is unlikely that the tissue energy supply would be sufficiently depleted under anaerobic conditions to stop ethylene production completely. On the other hand,

![Figure 3](image-url)

**Fig. 3.**—Ethylene production and oxygen consumption as a function of $pO_2$. Measurements were made over a 1-hr period after the gas mixture had been vacuum-impregnated into McIntosh apple tissue sections. All mixtures contained only $O_2$ and $N_2$; control tissue was treated with 80 per cent $N_2 - 20$ per cent $O_2$. The rates of both ethylene production and oxygen consumption of the controls are represented as 100 per cent. The ethylene curve (triangles) has not been corrected for the small ethylene production which occurs in the absence of $O_2$. 
ethylene is produced only during the maturation period when apple tissue has a cyanide-insensitive respiration, and ethylene production has been found to be unaffected by cyanide. The evidence at hand supports the idea that a cyanide-insensitive terminal oxidase of relatively high oxygen affinity is involved both in respiration and in ethylene production.

The data in Figure 3 show that there is a very slight production of ethylene during a 1-hr exposure to anaerobic conditions. The time course of this production was studied by placing plugs of tissue in syringes, evacuating to about 1 mm pressure, and then admitting nitrogen. Results are shown in Figure 4, curve A. A very small amount of ethylene is made for about 15 min after oxygen has been removed; then production of the gas ceases indefinitely. Since the preparations for this experiment required at most 2 min, the curve cannot be displaced more than 1 or 2 min along its axis from the exact moment at which anaerobic conditions were obtained. Although the small trace of ethylene produced after the onset of anaerobiosis might be accounted for by residual oxygen in the tissue, it is unlikely that very much oxygen remained and other evidence will be cited which supports the view that this ethylene was actually produced in the absence of oxygen.

Despite the fact that ethylene production soon ceases in nitrogen, some other reactions preliminary to ethylene formation apparently continue anaerobically.

![Figure 4](image-url)

**Fig. 4.**—Effect of nitrogen treatment on ethylene production by tissue sections. The values on the graph are average figures compiled from many experiments with McIntosh apple tissue sections. Control curve: production of ethylene by sections kept under atmospheric conditions for 4 hr before experiment. Curve A: ethylene production immediately after tissue was placed under nitrogen. Curve B: ethylene production by tissue previously stored under nitrogen for 4 hr, and then replaced in air. Curve C: in these experiments the tissue had been stored in nitrogen for 4 hr, placed in air for 5 min, re-evacuated, and returned to nitrogen, after which the ethylene production was recorded.
at a substantial rate. Thus, apple tissue which had been allowed to remain under nitrogen for 4 hr showed an accelerated rate of ethylene production when it was evacuated and the nitrogen replaced by air (Fig. 4, curve B). In Figure 4, the control curve represents the behavior of sections which had been cut from the same apple and allowed to remain in room air for 4 hr before being tested. A few apple plugs were assayed immediately after removal from the fruit, and they showed the same rate of ethylene production as the tissue used in deriving the control curve. Hence the high rate of ethylene production displayed by nitrogen-treated tissue is not the result of a decreased control rate. It should be noted that this increase in rate does not occur after the removal of some other inhibitors. For instance, high concentrations of carbon dioxide will retard ethylene production, an 80 per cent CO₂—20 per cent O₂ mixture giving about 40 per cent inhibition. However, after 4 hr, when the mixture is replaced with 20 per cent O₂—80 per cent N₂ the rate of ethylene production immediately returns to the control level, but not above it. It appears that prolonged nitrogen treatment causes the accumulation of some material (presumably a reduced compound), which can be converted to ethylene very rapidly in the presence of oxygen.

This same type of behavior is exhibited by whole apples. The internal concentration of ethylene in an apple can be readily determined by extracting a small sample of air from the fruit with a hypodermic syringe; this sample can then be gas-chromatographed. If the extraction is carried out under water, considerable accuracy can be obtained (as for example in Fig. 1, top). When whole apples were evacuated in a desiccator, and replaced in air, the internal ethylene accumulated at a decreasing rate as shown in Figure 5, Controls. When the apples were re-evacu-

FIG. 5.—Effect of nitrogen treatment on ethylene production by whole apples. When McIntosh apples were evacuated and returned to atmospheric conditions, the internal ethylene concentration increased along the curve shown as Controls. The fruits were re-evacuated at the last point indicated on this curve, after which the internal ethylene accumulated along an identical curve. Other apples were vacuum-impregnated with nitrogen and left for 4 hr. The fruits were then evacuated and placed in air, after which the internal ethylene concentration rose along the curve marked Sample 1. When the tissue was re-evacuated after 65 min, the internal concentration now increased at the same rate as the control, Sample 2. Each curve is an average compiled from the behavior of three apples. Four hours after the last recorded points, the samples had reached an internal concentration of 0.0471 per cent, and the controls 0.0572 per cent.
ated, the ethylene again accumulated along an identical curve. The curve marked Controls in Figure 5 actually comprises points from before and after the re-evacuation. However, if the apples had been stored under nitrogen for 4 hr and then evacuated and returned to atmospheric conditions, a remarkably high rate of ethylene accumulation resulted (Fig. 5, Sample 1). When, after 65 min, the treated apples were re-evacuated, they then showed a low rate of ethylene accumulation equal to that of the controls (Fig. 5, Sample 2). This experiment has been carried out three times with virtually identical results. It is evident that nitrogen storage leads to an accelerated rate of ethylene production when aerobic conditions are subsequently reinstated.

The experiments shown in Figures 4 and 5 indicate that the initial acceleration of ethylene production occurring after nitrogen storage is short-lived, and it is followed by a transient period in which little or no ethylene is produced. In Figure 4 the decline takes place at about 75 min, when the rate falls essentially to zero before it recovers to what appears to be a control value. In Figure 5, after about 30 min the internal concentration of ethylene begins to fall in Sample 1, before it again rises at about 55 min. The discrepancy in timing between the whole fruit and tissue plugs is not understood, but in general the results show that some precursor can be converted anaerobically to a substance which is oxidized to ethylene, that this precursor becomes depleted in nitrogen, and that it is replaced only slowly in air.

Even if apple tissue which has been in nitrogen for 4 hr is exposed to air for only 5 min, it can go on to produce ethylene for about 60 min when it is replaced under anaerobic conditions. The initial rate of ethylene production of tissue so treated, as shown in Figure 4, curve C, must be about the same as in control tissue. This supports the preceding conclusion that a substance accumulates under nitrogen, and it can then be oxidatively converted to ethylene. In addition, curve C indicates that the terminal step or steps in ethylene production must be nonoxidative. The oxidative “reaction” must be extremely rapid since it can supply sufficient oxidized precursor in 5 min to support subsequent ethylene production for about 60 min. Normally, the concentration of the oxidized precursor present in the tissue must be so low that it can be exhausted within 15 min in the absence of oxygen (see Fig. 4, curve A).

4 Experiments with labeled precursors: Experiments with carbon and tritium labeled compounds will be described in a future publication, but a few results are included here because of their relationship to the scheme of ethylene production which can be derived from the experiments cited above.

If tritiated water is admitted into apple tissue, its label becomes incorporated very rapidly into the ethylene. Since the dissolving of ethylene in tritiated water was found to produce little or no exchange, it follows that the incorporation into ethylene in the tissue is metabolic. In the same period of time which is required for complete equilibration of the added isotope with the tissue water (about 3 min), the ethylene was found to reach approximately the same specific activity as the isotopic water within the tissue. Water must therefore be able to enter and leave one or more precursors of ethylene in the position which will ultimately correspond to the ethylenic double bond.

The role of reducing hydrogens in ethylene synthesis was studied by means of tritiated glycerol. The isotope contained a nonlabile-tritium in the 2-carbon posi-
tion; this is the position known to yield reducing hydrogen when glycerol is metabolically dehydrogenated. The glycerol was soaked into large plugs of apple tissue for 60 min under anaerobic conditions in order to prevent its metabolism during the feeding period. After the sections had been exposed to air for a further 15 min, a significant transfer of label to malic and succinic acids and sugars had occurred; in one instance the ethylene was also radioactive but somewhat low in specific activity, while in a second experiment no measurable activity could be detected in the ethylene at all. Malic acid, which represents a large metabolic pool in apple tissue, has three nonlabile hydrogens and two of these are in a position where known reactions would result in an extensive dilution with water. Yet this substance had a higher specific activity (620 cpm/μM) than the ethylene (0 and 550 cpm/μM), even though the latter compound is present in minute amounts and contains four nonlabile hydrogens. It follows that, in the synthesis of ethylene, if reductions (involving hydrogen atoms which can be even indirectly derived from the 2-carbon position of glycerol) occur, they must take place prior to the hydration-dehydrogenation reaction indicated in the preceding paragraph.

Discussion.—The data presented in the preceding sections are summarized in Figure 6 in the form of a sequence of reactions leading to the production of ethylene. The evidence for the various steps is as follows:

1. Compound A accumulates under nitrogen, by one or more reactions probably involving reductions. The existence of this substance is revealed by the accelerated rate of ethylene production observed when oxygen is re-admitted (Figs. 4 and 5).

2. The almost immediate cessation of ethylene production under anaerobic conditions indicates that ethylene production is in some way linked to the utilization of atmospheric oxygen. Although ethylene production and oxygen uptake show a very similar dependence upon oxygen partial pressure (Fig. 3), it is unlikely that the correlation is mediated through energy production. In the case of ethylene, a cyanide-insensitive terminal oxidase may accept electrons derived from a dehydrogenation step, here pictured as the conversion of A to B. The same terminal oxidase may also accept electrons from respiration.

3. Evidence for a reversible dehydration step which cannot be followed by a hydrogenation or reversible dehydrogenation, was derived from experiments with tritiated water and tritiated glycerol. This is represented as the conversion of compound B to C.

4. The final step or steps in ethylene production must be nonoxidative since tissue treated with nitrogen for 4 hr is able to produce ethylene anaerobically after a brief exposure to oxygen (Fig. 4, curve C). The oxidative conversion of A to B must be extremely rapid, for when the short oxygen treatment is terminated, a sufficient quantity of oxidized precursor has been produced to sustain ethylene production for a considerable time.

5. It should be noted that A, B, and C are all nonvolatile.

Summary.—The production of ethylene by sections of apple tissue has been
investigated by a new and precise method. The optimal temperature for the process is 32°C, and above this ethylene production falls off rapidly. The heat inactivation slowly disappears when the tissue is exposed to lower temperatures. Ethylene production and oxygen consumption show almost identical dependence upon oxygen tension, the half maximum value for both being reached at 1.5–2.0 per cent O₂. Although the synthesis of ethylene ceases almost immediately under anaerobic conditions, a precursor accumulates which can be rapidly oxidized in air with the production of ethylene. Tritiated water transfers its label to ethylene in such a manner as to indicate that one of the terminal steps in the process is a reversible dehydration. The final reaction leading to ethylene does not require the participation of oxygen. A scheme for ethylene production is proposed in which one or more reductions are followed by a rapid oxidation, a reversible dehydration, and a nonoxidative terminal step.

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8 Nelson, R. C., Ph.D. Dissertation, University of Minnesota, 1938.

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**PHOTOCONTROL OF PLANT DEVELOPMENT**

**BY THE SIMULTANEOUS EXCITATIONS OF TWO INTERCONVERTIBLE PIGMENTS**

By S. B. HENDRICKS and H. A. BORTHWICK

U.S. DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH SERVICE, PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND

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Flowering, seed germination, stem elongation, and many other aspects of growth and development of seed plants are influenced by a reversible photoreaction. This reaction can be written:

\[
\text{PH}_2 + A \xrightarrow{\text{6,600 A max}} P + \text{AH}_2
\]

\[
\text{PH}_2 + A \xrightarrow{\text{7,350 A max}} P + \text{AH}_2
\]