Ethylene Production and Respiration in Aging Leaf Segments and in Disks of Fruit Tissue of Normal and Mutant Tomatoes

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ABSTRACT

Leaf segments of tomato plants (Lycopersicon esculentum Mill.) of a normal strain and of two nonripening mutants rin and nor were aged in darkness. Respiration in leaf segments of all strains followed a climacteric-like pattern which was accompanied by a similar pattern of ethylene production. L-Methionine-U-14C vacuum-infiltrated into leaf segments at the beginning of the climacteric-like rise in respiration was metabolized to ethylene and CO2 during the subsequent 48 hours to about the same extent in all strains. Pericarp disks of immature fruits of all strains also metabolized L-methionine-U-14C to ethylene and CO2 to about the same extent during the first 48 hours following cutting and vacuum infiltration. Conversion of methionine to ethylene in disks was much more efficient than in aging leaf segments. The apparent capacity for increased production of ethylene in aging leaf segments and in response to wounding in pericarp disks of rin and nor is contrasted with the absence of a respiratory climacteric and an associated large increase in ethylene production during natural aging of intact fruits of these two strains.

Recently Zobel (11) reported that the diageotropic mutant of tomato requires exogenous ethylene for normal vegetative growth and development, whereas fruit maturation and ripening and production of ethylene by flowers is normal. Zobel suggested that his observations may indicate a possible divergence of mechanisms controlling ethylene synthesis in different tissues. Two mutants, rin and nor, which grow normally but produce nonripening fruits have also been reported (7, 8). Physiological studies with rin have shown that developing fruits contain low levels of ethylene similar to those in a normal strain and eventually turn yellow on the plant or following detachment (3, 5). Even when stored for long periods at temperatures suitable for normal ripening, rin fruits do not undergo the respiratory climacteric and the associated large increase in ethylene production found during ripening in normal tomato fruits (3). Previous work

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2. This research was completed while W. B. McGlasson was on leave from the Plant Physiology Unit, Commonwealth Scientific and Industrial Research Organization, Division of Food Research, and School of Biological Sciences, Macquarie University, North Ryde 2113, Sydney, Australia.

MATERIALS AND METHODS

Tomatoes (Lycopersicon esculentum Mill.) were grown in a greenhouse. The strains used were 'Rutgers' and partially isogenic strains of rin and nor developed by backcrosses to Rutgers. Seed of these strains was supplied by Associate Professor E. C. Tigchelaar of Purdue University. Samples (about 10 g) of leaf segments were harvested at random from young fully expanded tomato leaves and placed immediately in glass jars, which were wrapped in foil to exclude light, and ventilated continuously with CO2 and ethylene-free humidified air at 20 C. The fruits used in the tracer experiment were picked at about 70% of the total growth period from uniform populations (5). Disks, diameter 2.5 cm, were cut aseptically (4) and held in sterile glass jars ventilated continuously with CO2 and ethylene-free air at 20 C. CO2 and ethylene production by the leaf segments and by fruit disks were measured respectively with an infrared gas analyzer and a gas chromato- graph equipped with a flame ionization detector.

L-Methionine-U-14C, specific radioactivity 260 mCi/mmmole, was obtained from Schwarz/Mann Laboratories, N. Y. Leaf segments were removed from the glass jars on the 1st day of the climacteric-like rise in CO2 production and vacuum-infiltrated at 25 cm Hg for 1 min with an aqueous solution of methionine containing about 0.4 μCi/ml. After infiltration the leaf segments were lightly blotted with tissue paper and returned to glass jars which were rewrapped in foil. Vacuum infiltration slightly depressed the rise in CO2 and ethylene production. The fruit disks were similarly vacuum-infiltrated with an aqueous solution containing 0.8 μCi/ml of methionine.

The effluent air streams from the jars of labeled leaf segments and fruit disks were passed through a solution of mercuric perchlorate at 0 C to trap ethylene and then through a solution of ethanolic methoxyethanol to trap CO2 (6, 9). Following addition of scintillants to these solutions (6, 9), radioactivity was measured in a liquid scintillation spectrometer. A count was made on a second aliquot of the mercuric perchlorate solution.
following addition of lithium chloride to release absorbed ethylene. The difference in counting efficiency was taken as a measure of the incorporation of 14C into ethylene.

RESULTS AND DISCUSSION

Figure 1 shows that the patterns of CO2 and ethylene production by aging leaf segments of normal, rin, and nor plants were similar. Both CO2 and ethylene production declined during the first 3 or 4 days after harvest. Depending on the age of the leaves at harvest, there followed a period of steady production of both gases of 1 or more days duration until the onset of the climacteric-like rise in CO2 production. This rise was accompanied by a gradual rise in ethylene production. Peak production of both gases was reached at about the same time. Subsequent to these peaks, ethylene production declined rapidly but CO2 production declined more gradually. A mottled pattern of yellowing was observed within 2 days after the peak in CO2 production. Decay usually did not become apparent until about 3 days after the peak. Subsequent to the completion of the experiments with leaf segments, it was found that about one-fourth of the plants in the nor planting were normal. Since the rates of CO2 and ethylene production shown for Rutgers and nor were similar (Fig. 1), it is logical to assume that the true rates of production of these gases by leaf segments from nor plants were normal.

Table I. Evolution of 14C2H4 and 14CO2 by Leaf Segments Vacuum-infiltrated with L-Methionine-U-14C

Three bulk samples (10 g) of each strain were vacuum infiltrated at the beginning of the climacteric-like rise in CO2 production. The statistical limits are estimates of the standard deviations of the population.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-12 hr</td>
</tr>
<tr>
<td>Rutgers</td>
<td>26 ± 38</td>
</tr>
<tr>
<td>rin</td>
<td>22 ± 11</td>
</tr>
<tr>
<td>nor</td>
<td>47 ± 6</td>
</tr>
</tbody>
</table>

Table II. Evolution of 14C2H4 and 14CO2 by Disks of Fruit Pericarp Tissue Vacuum-infiltrated with L-Methionine-U-14C

A single disk (2.5 cm diam) cut from each of three immature fruits of each strain was vacuum-infiltrated immediately after cutting. The data shown are ratios of 14C2H4/14CO2 evolved multiplied by 100. The statistical limits are estimates of the standard deviations of the population.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-4 hr</td>
</tr>
<tr>
<td>Rutgers</td>
<td>8.9 ± 3.4</td>
</tr>
<tr>
<td>rin</td>
<td>10.0 ± 3.4</td>
</tr>
<tr>
<td>nor</td>
<td>11.3 ± 8.6</td>
</tr>
</tbody>
</table>

L-Methionine-U-14C was vacuum-infiltrated into leaf segments at the beginning of the climacteric-like rise in respiration to determine whether there were any substantial differences in the metabolism of methionine between strains. Methionine was readily metabolized but the actual amounts of radioactivity evolved as CO2 and ethylene varied between replicates, presumably because of variability in uptake of methionine. To facilitate comparison between strains the ratios of labeled ethylene to labeled CO2 evolved were calculated for each 12-hr collection period during the first 48 hr following infiltration (Table I). This method of presenting data was used previously in a study with green banana fruit tissue (9) and was based on the finding that C1 of methionine is readily converted to CO2 and C3 and 4 are specifically converted to ethylene but C2 is retained in the tissue (1). There is also clear evidence for apple tissue that C5 is not converted to ethylene and is mostly retained in the tissue although some may be released as CO2 (10). It was proposed that if this pattern of metabolism applies in tomato leaf segments any differences in metabolism between normal and mutant strains might be revealed by changes in the ratios 14C2H4/14CO2. Table I shows that the ratios were similar for each strain during each collected period. Calculations of the ratios 14C2H4/14CO2 from actual measurements of ethylene and CO2 production during the 48 hr following infiltration gave values ranging from 41 × 10^-4 to 71 × 10^-4. Thus the conversion of labeled methionine to 14C2H4 relative to 14CO2 (Table I) was about 25 times more efficient than predicted from measurements of ethylene and CO2 production.

A similar study was made of the conversion of labeled methionine to ethylene and CO2 by freshly cut fruit pericarp disks of the three strains. Table II shows that the highest values for the ratios 14C2H4/14CO2 evolved were found for that period 4 to 12 hr after cutting. Measurements of ethylene and CO2 production by separate bulk samples each of three disks during the first 48 hr after cutting also showed that the responses to cutting were
similar in the three strains. Calculation of the ratios $^{14}$C$_2$H$_4$/
$^{14}$CO$_2$ from these measurements gave the following values, 
$5 \times 10^{-4}$ at 4 hr, $1.4 \times 10^{-4}$ at 12 hr, and $1 \times 10^{-4}$ at 48 hr.
Comparison of these ratios with those in Table II shows that the
conversion of L-methionine-U-$^{14}$C to ethylene relative to CO$_2$
was much more efficient in disks of fruit tissue than in leaf seg-
ments. In addition to conversion into CO$_2$ and ethylene there was
a considerable conversion of L-methionine-U-$^{14}$C into unknown
compounds which were trapped by mercuric perchlorate but not
released upon addition of lithium chloride. These unknown com-
ounds contained about 25% as much radioactivity as was
recovered in CO$_2$. 

Our data clearly show that there are no substantial differences
between the normal, rin, and nor strains in capacity for the pro-
duction of ethylene by either aging leaf tissue or by freshly cut
disks of fruit tissue. Herner and Sink (3) have previously reported
that wounding stimulates ethylene production by rin fruit tissue.
We have now shown that there is an active conversion of methi-
onine to ethylene in wounded fruit tissue of both rin and nor,
quantitatively similar to that in wounded normal fruit tissue. The
production of ethylene in response to wounding contrasts with the
lack of a natural respiratory climacteric and parallel increase in ethylene production during aging in whole rin fruit
(3, 5), and the occurrence of only a very small rise in ethylene
production but no respiratory climacteric in intact aging whole
nor fruit (2). These apparent differences in ethylene metabolism
in aging leaves and freshly cut disks compared with intact aging
whole fruits support Zobel's (11) suggestion of a possible diver-
gence of mechanisms controlling ethylene synthesis in different
tissues. They also provoke further questions about the role of
ethylene in aging leaves and in wounding.

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CORRECTIONS

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Vol. 56: 332–334. 1975
Page 333, column 2, Table 1, should be corrected to read:

Table I. Germination Response of Pretreated Seed Batches to Light Treatments

<table>
<thead>
<tr>
<th>Seed Batch (Pretreatment)</th>
<th>Light Treatment</th>
<th>Germination (%)</th>
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</thead>
<tbody>
<tr>
<td>D</td>
<td>D</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>R/F</td>
<td>6</td>
</tr>
<tr>
<td>R</td>
<td>D</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>R/F</td>
<td>9</td>
</tr>
<tr>
<td>Cont R</td>
<td>D</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>R/F</td>
<td>25</td>
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<tr>
<td>F</td>
<td>D</td>
<td>1</td>
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<tr>
<td></td>
<td>R</td>
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<td>R</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>R/F</td>
<td>10</td>
</tr>
</tbody>
</table>

Vol. 56: 547–549. 1975
McGlasson, William B., B. W. Poovaiah, and Herbert C. Dostal. Ethylene Production and Respiration in Aging Leaf Segments and in Disks of Fruit Tissue of Normal and Mutant Tomatoes.
Page 548, column 2, Table 1 legend, should be corrected to read: Three bulk samples (10 g) of each strain were vacuum-infiltrated at the beginning of the climacteric-like rise in CO₂ production. The data shown are ratios of ¹⁴C₂H₄/¹⁴CO₂ evolved multiplied by 10,000. The statistical limits are estimates of the standard deviations of the populations.