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Ethylene Production and Respiration in Aging Leaf Segments and in Disks of Fruit Tissue of Normal and Mutant Tomatoes[1]

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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-^{14}C vacuum-infiltrated into leaf segments at the beginning of the climacteric-like rise in respiration was metabolized to ethylene and CO_2 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-^{14}C to ethylene and CO_2 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 in this laboratory (2) has shown that immature fruits of *nor* also fail to exhibit a respiratory climacteric. Yellowing in *nor* fruits is associated with a small rise in ethylene production to a peak value about one-eighth of that in normal fruit.

Since leaves are considered to be morphogenically homologous with fruits, it seemed of interest to compare that responses of aging leaf tissue of *rin* and *nor* with those of a normal strain. In this paper, we report the results of a comparative study of the patterns of respiration and ethylene production. We have also included data from single experiments in which L-methionine-U-^{14}C was fed to aging leaf tissue and to freshly cut disks of the pericarp of immature fruit of the three strains, since methionine has been shown to be the major precursor of ethylene in higher plants (10).

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 CO_2- 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 CO_2- and ethylene-free air at 20 C. CO_2 and ethylene production by the leaf segments and by fruit disks were measured respectively with an infrared gas analyzer and a gas chromatograph equipped with a flame ionization detector.

L-Methionine-U-^{14}C, specific radioactivity 260 mCi/mmole, 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 CO_2 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 CO_2 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 ethanolamine in methoxyethanol to trap CO_2 (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

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following addition of lithium chloride to release absorbed ethylene. The difference in counting efficiency was taken as a measure of the incorporation of ^{14}C into ethylene.

RESULTS AND DISCUSSION

Figure 1 shows that the patterns of CO_2 and ethylene production by aging leaf segments of normal, *rin*, and *nor* plants were similar. Both CO_2 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 CO_2 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 CO_2 production declined more gradually. A mottled pattern of yellowing was observed within 2 days after the peak in CO_2 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 CO_2 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.

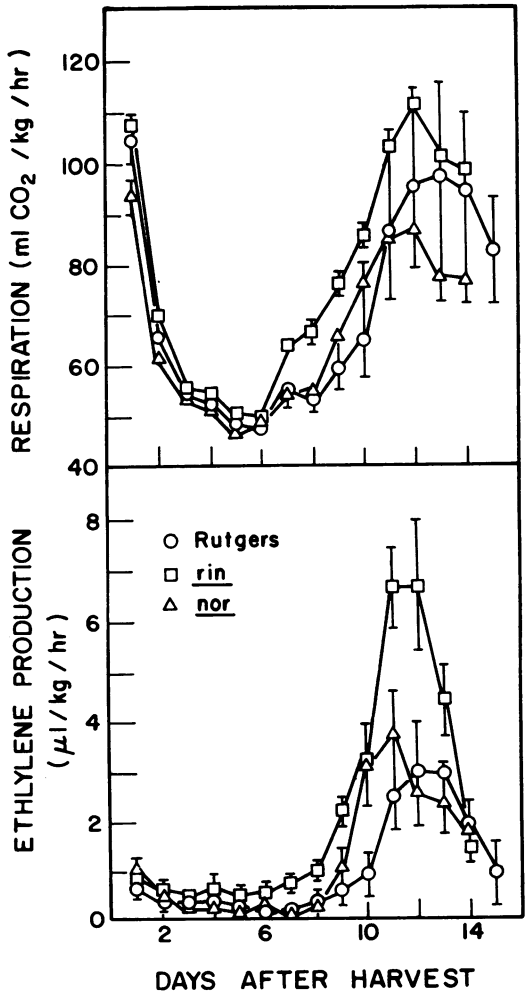

FIG. 1. Average rates of CO_2 and ethylene production by leaf segments of Rutgers, *rin*, and *nor*. The vertical bars indicate estimates of the standard deviations of the population ($n^1 = 3$).

Table I. *Evolution of $^{14}C_2H_4$ and $^{14}CO_2$ by Leaf Segments Vacuum-infiltrated with* L-*Methionine-U-^{14}C*

Three bulk samples (10 g) of each strain were vacuum infiltrated at the beginning of the climacteric-like rise in CO_2 production. The statistical limits are estimates of the standard deviations of the population.

Strain	Time Interval			
	0–12 hr	12–24 hr	24–36 hr	36–48 hr
Rutgers	26 ± 38	28 ± 12	19 ± 18	27 ± 5
rin	22 ± 11	28 ± 11	26 ± 13	30 ± 11
nor	47 ± 6	31 ± 5	33 ± 3	

Table II. *Evolution of $^{14}C_2H_4$ and $^{14}CO_2$ by Disks of Fruit Pericarp Tissue Vacuum-infiltrated with* L-*Methionine-U-^{14}C*

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 $^{14}C_2H_4/^{14}CO_2$ evolved multiplied by 100. The statistical limits are estimates of the standard deviations of the population.

Strain	Time Interval				
	0–4 hr	4–12 hr	12–14 hr	24–36 hr	36–48 hr
Rutgers	8.9 ± 3.4	24.0 ± 8.3	21.3 ± 7.4	21.2 ± 3.7	17.4 ± 5.7
rin	10.0 ± 3.4	28.3 ± 8.1	20.8 ± 3.0	20.8 ± 2.5	17.6 ± 1.2
nor	11.3 ± 8.6	19.8 ± 4.6	17.0 ± 1.5	16.4 ± 4.8	16.4 ± 7.1

L-Methionine-U-^{14}C 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 CO_2 and ethylene varied between replicates, presumably because of variability in uptake of methionine. To facilitate comparisons between strains the ratios of labeled ethylene to labeled CO_2 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 CO_2, 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 CO_2 (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 $^{14}C_2H_4/^{14}CO_2$. Table I shows that the ratios were similar for each strain during each collected period. Calculations of the ratios $^{14}C_2H_4/^{14}CO_2$ from actual measurements of ethylene and CO_2 production during the 48 hr following infiltration gave values ranging from 41×10^{-6} to 71×10^{-6}. Thus the conversion of labeled methionine to $^{14}C_2H_4$ relative to $^{14}CO_2$ (Table I) was about 25 times more efficient than predicted from measurements of ethylene and CO_2 production.

A similar study was made of the conversion of labeled methionine to ethylene and CO_2 by freshly cut fruit pericarp disks of the three strains. Table II shows that the highest values for the ratios $^{14}C_2H_4/^{14}CO_2$ evolved were found for that period 4 to 12 hr after cutting. Measurements of ethylene and CO_2 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 $^{14}C_2H_4/^{14}CO_2$ from these measurements gave the following values, 5×10^{-4} at 4 hr, 1.4×10^{-4} at 12 hr, and 1×10^{-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 segments. 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 compounds 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 production 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 methionine 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 divergence 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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