

Comparison of Propylene-induced Responses of Immature Fruit of Normal and *rin* Mutant Tomatoes^{1,2}

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ABSTRACT

Continuous application of propylene to 40 to 80% mature fruits of normal tomato strains (*Lycopersicon esculentum* Mill.) advanced ripening in fruits of all ages by at least 50%. Although preclimacteric respiration was stimulated by propylene treatment, there was no concomitant increase in ethylene production. Once ripening commenced, the rates of endogenous ethylene production were similar in both propylene-treated and untreated fruits. Continuous exposure to propylene also stimulated respiration in immature fruits of *rin*, a nonripening mutant. Although respiration reached rates similar to those during the climacteric of comparable normal fruits there was no change in endogenous ethylene production which remained at a low level. Internal ethylene concentrations in attached 45 to 75% mature fruits of *rin* and a normal strain were similar. It is suggested that the onset of ripening in normal tomato fruit is not controlled by endogenous ethylene, although increased ethylene production is probably an integral part of the ripening processes.

In commercial practice, tomato fruits are harvested at the mature green stage to facilitate shipment. Satisfactory non-destructive indices of maturity are not available for tomatoes, and commercial consignments usually comprise fruits of a wide range of physiological ages. In other immature climacteric fruits, e.g., bananas and melons, uniform ripening can be achieved by treating the fruit with ethylene (8). However, treatment of immature tomato fruits with ethylene even at high concentrations, does not result in uniform ripening, although ripening in individual fruits is advanced. Lyons and Pratt (5) showed that continuous treatment with 1000 μ l/l ethylene in air reduced the time to ripen by about one-half in fruit picked at from 64 to 93 of the total growth period.

At 20 C, the commencement of natural ripening in tomato fruits is indicated by a simultaneous increase in respiration and ethylene production. Red or orange pigments usually appear

1 to 2 days later. The role of endogenous ethylene in ripening of climacteric fruits has been debated for many years. Presently, most workers suggest from the accumulated evidence that ethylene plays a specific role in the initiation of ripening in most climacteric fruits (6, 8). Stronger evidence for the involvement of ethylene production in the ripening processes, once initiated, can be deduced from several recent studies. It has been shown for bananas that ethylene is required for normal integration of various ripening processes (4, 9). Other workers have demonstrated that an autocatalytic increase in ethylene production in conjunction with increased respiration and other indications of ripening is a characteristic of climacteric fruits (3, 7). If enhanced ethylene production acts normally to initiate and integrate the ripening processes, a major problem is to determine how ethylene evolution is controlled. We have approached this problem by examining the responses of immature tomato fruit to treatment with propylene. Propylene, an active analogue of ethylene, was substituted for ethylene because it enables measurement of endogenous ethylene production by treated fruits (3, 7, 12). Our studies included work with immature fruit of the mutant type *rin*. *Rin* fruit have been reported to develop and mature normally but to remain green when normal fruit turn red (11). Little or no change in ethylene or CO₂ production has been reported in *rin* fruit monitored for up to 120 days after harvest even though a gradual softening and yellowing occurred during this period (3). While a climacteric-like increase in respiration can be induced in *rin* fruit by a 2- to 4-day exposure to ethylene or propylene, ethylene production remains low unless the fruit are injured (3). Although yellowing and softening occur in both ethylene-treated and untreated *rin* fruit, the fruit do not develop the flavor and texture characteristic of normal cultivars. The data reported in this paper lead us to re-examine the role of endogenous ethylene in the initiation of natural ripening in the tomato fruit and to reinforce the view that increased ethylene production is an integral part of the ripening processes.

MATERIALS AND METHODS

Tomatoes (*Lycopersicon esculentum* Mill.) were grown in a greenhouse. The cultivars used were an F1 ('Purdue 123' × 'Hoosier X1-1,' bred for commercial production by Davis Gardens Inc., Terre Haute, Ind.), 'Rutgers,' and a partially isogenic strain of the *rin* mutant developed by two backcrosses to 'Rutgers.' Uniform populations of fruits were produced by restricting the fruit load per plant (5). The fruits were picked at various intervals after anthesis. To facilitate comparisons between fruit populations and cultivars, the ages of the fruit at picking were expressed as percentages of the total growth period, 100% being the average time after anthesis for the appearance of red or pink color of representative fruits of each population still attached to the plant (5). *Rin* fruits were

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picked on the same time scale as were Rutgers, since it was previously established that fruits set on the same date grew to full size in about the same time in both strains.

After harvest the fruits were weighed, then dipped for 1 min in a fungicidal suspension composed of 0.1% (w/v) Benomyl (methyl 1-butylcarbomyl-2-benzimidazolcarbamate, DuPont), 0.2% (w/v) Botran (2-6-dichloro-4-nitroaniline, Upjohn), and 0.025% (w/v) of a nonionic wetting agent. After dipping, the fruits were enclosed individually in glass jars which were ventilated continuously with humidified CO₂-free air, or humidified CO₂-free air containing 300 to 1000 μ l/l propylene, at about 1 l/hr · 100 g fruit tissue.

Air streams containing propylene were prepared by mixing metered flows of humidified CO₂-free air and of a purchased mixture of 4% (v/v) propylene in N₂. CO₂ and ethylene production levels were measured daily with an infrared gas analyzer and a gas chromatograph, respectively. The gas chromatograph was equipped with a flame ionization detector. The hydrocarbons were separated on a 152 × 0.32 cm column of 60 to 80 mesh Al₂O₃ at 80 C. The lowest concentration of ethylene that could be measured was 0.005 μ l/l in a 1-ml sample. The retention times were 1 and 4 min for ethylene and propylene, respectively. To measure internal concentrations of ethylene in developing fruit, samples of gas were withdrawn by syringe from fruit immersed in H₂O.

RESULTS

To establish the concentrations of propylene required to produce the maximum advancement of ripening, Rutgers fruits, picked at 43 and 61% of the total growth period were treated continuously with 300, 500, and 1000 μ l/l. The onset of the exponential rise in endogenous ethylene production was used as a parameter of ripening. Table I shows that all three concentrations of propylene were similarly effective in advancing ripening. However, in supplementary studies with immature fruits of field grown cv. Heinz 1439, 300 μ l/l was less effective than higher concentrations. Therefore, 500 μ l/l was adopted as a standard treatment to ensure the presence of a moderate excess of the gas.

Figure 1 shows the response of the hybrid cultivar to propylene as a function of age at harvest. CO₂ production by untreated 40 and 60% mature fruit declined for 1 or 2 days after picking, rose to a peak 4 to 6 days after picking and then declined to a preclimacteric minimum. These changes which also have been observed consistently in Rutgers (Fig. 2) were not accompanied by changes in ethylene production rates. Treatment with propylene caused an increase in CO₂ produc-

Table I. Comparison of Effectiveness of Three Concentrations of Propylene in Advancing Ripening in Immature Rutgers Fruits of 2 Ages

	Days to Ripen ¹	
	43% mature	61% mature
Air	25.0 ± 1.2 ²	18.3 ± 2.0
Propylene		
300 μ l/l	12.0 ± 0	9.3 ± 0.8
500 μ l/l	13.3 ± 1.5	7.0 ± 0.8
1000 μ l/l	10.1 ± 0.8	8.3 ± 0.8

¹ The onset of the exponential rise in ethylene production was used as a parameter of ripening.

² The statistical limits are estimates of the standard deviations of the population ($n^1 = 3$).

Table II. Internal Concentrations of Ethylene in Attached Fruits of Rutgers and *rin*

Total Growth	Rutgers	<i>rin</i>
$\%$	ethylene μ l/l	
45	0.151 ± 0.054 ¹	0.132 ± 0.041
64	0.104 ± 0.022	0.076 ± 0.026
75	0.061 ± 0.014	0.044 ± 0.010
143		0.151

¹ The statistical limits are estimates of the standard deviations of the population ($n^1 = 3$). The 143% sample comprised only two fruits.

tion which, in 60 and 80% fruit, merged into the respiratory climacteric. These initial increases in respiration were not accompanied by increased ethylene production which remained low until the onset of ripening.

As in the untreated fruits, ethylene production in propylene-treated fruit rose abruptly with the onset of ripening. Color development was observed on the 2nd day after the beginning of the rise in ethylene production in both control and treated fruits. Treatment with propylene reduced the time between harvest and ripening of fruits of all ages by at least one-half. (Fig. 1 and Table I).

The responses of 43% mature *rin* and Rutgers fruits to propylene are compared in Figure 2. For the first few days the rates of respiration in untreated fruits and the respiration responses to propylene were similar in both strains. Rutgers fruits subsequently ripened and conformed to the patterns described for the hybrid cultivar. In contrast, respiration rates in untreated *rin* fruits declined gradually to a low level and ethylene production remained barely measurable. These fruits eventually turned yellow. In treated *rin* fruits respiration continued at a high level for several days past the time of the post-climacteric decline in respiration in treated Rutgers fruits but ethylene production remained at a barely measurable level. The appearance of yellow color was advanced by propylene treatment.

Table II shows that the internal concentration of ethylene in attached fruits of Rutgers and *rin* were similar. The 143% fruits of *rin* had been left on the plants for about 19 days after comparable Rutgers fruits had reached the 100% stage and at the time of sampling had begun to turn yellow.

DISCUSSION

Propylene at 500 μ l/l applied continuously to immature fruits (40–80% mature) of normal tomato strains advances ripening in all stages by at least 50%. The close agreement between this finding and that of Lyons and Pratt (5) for ethylene indicates that the responses to ethylene and propylene are equivalent. Our studies provide the additional information that propylene treatment of normal fruit does not stimulate ethylene production during the preclimacteric period, and furthermore, the rise in endogenous ethylene production in propylene-treated fruit is normal in rate and timing in relation to color development (Fig. 1).

The data reviewed by Pratt and Goeschl (8) suggest that the autocatalytic burst in ethylene production, the respiratory climacteric, and other concomitant changes which comprise ripening in climacteric fruits result from either a gradual increase in cellular sensitivity to endogenous ethylene, a gradual increase in endogenous ethylene to a threshold concentration, or a combination of these two possibilities. If sufficient exogenous ethylene (or propylene) is added to immature

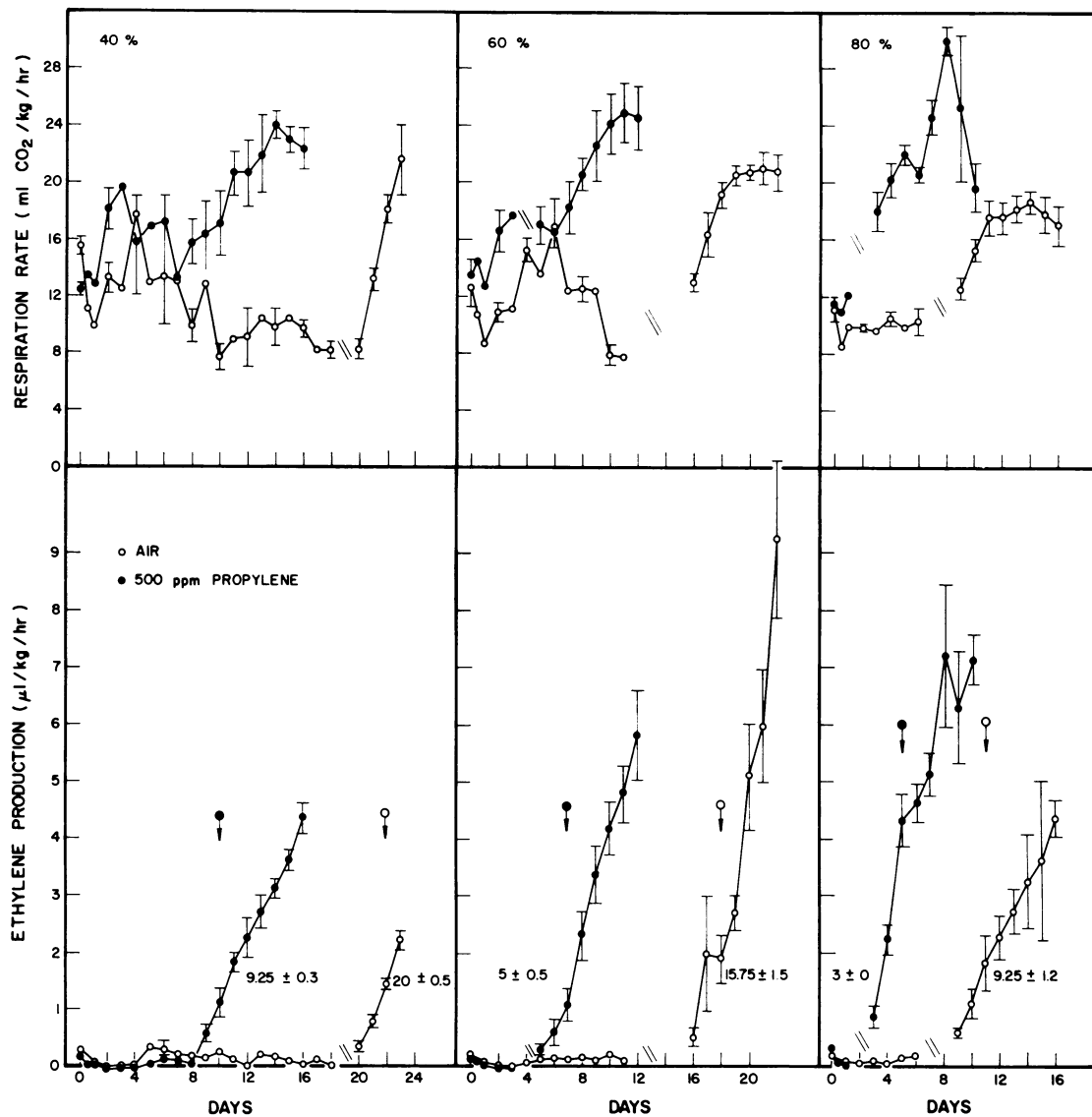


FIG. 1. Average rates of CO_2 and ethylene production by propylene-treated and untreated fruits of F1 tomatoes (Purdue 123 \times Hoosier X1-I) at three stages of maturity. The vertical bars indicate estimates of the standard deviations of the population ($n^1 = 3$). For clarity, estimates for alternate points on the curves were omitted. The arrows indicate the average time at which the fruits showed the first detectable red color and the numbers, with estimates of the standard deviations of the population, indicate the average elapsed time to the onset of the rapid rise in ethylene production. To provide a more accurate presentation of the rates of CO_2 and ethylene production during ripening, composite curves were prepared by matching the data for individual fruits in each treatment. The day of the first significant increase in ethylene production was taken as day 1 of ripening. The double slashes mark the breaks in the curves necessitated by variation in time taken by individual fruits to initiate ripening.

tissue, the system responsible for the natural tolerance to this gas should be overwhelmed and fruits of all physiological ages should commence to ripen at about the same rate. Such an observation has been made for several climacteric fruits (8). However, in tomato fruits of normal strains the tolerance of immature fruits cannot be completely overwhelmed by ethylene or propylene treatment.

Ethylene or propylene treatment stimulates respiration in *rin* fruits as it does in preclimacteric fruits of normal ripening strains (Fig. 2) (3). Even after prolonged periods of treatment or storage, no increases in ethylene production have been observed. Nevertheless, *rin* fruit tissues are capable of ethylene production. Table II shows that the internal levels of ethylene in developing *rin* and Rutgers fruits are similar and Herner and Sink (3) reported that wounding stimulated ethylene production by *rin* fruit tissue. McMurchie *et al.* (7) showed that

treating banana fruit with propylene stimulated a typical respiratory climacteric and a rise in ethylene production. In contrast, lemons and oranges, examples of nonclimacteric fruits, showed only a climacteric-like rise in respiration in response to propylene. These results were considered to support previous suggestions that the critical difference between climacteric and nonclimacteric fruits rests in their relative abilities to produce ethylene in response to low concentrations of ethylene (2, 10). On the basis of the similarity of the responses of *rin* and citrus fruits to propylene, we concur with the conclusion of Herner and Sink (3) that *rin* fruit behave as a nonclimacteric tissue.

From these comparisons of the responses of different fruits, we suggest that the onset of ripening in normal strains of tomato fruit is not controlled by endogenous ethylene, although increased ethylene production appears to be an essential com-

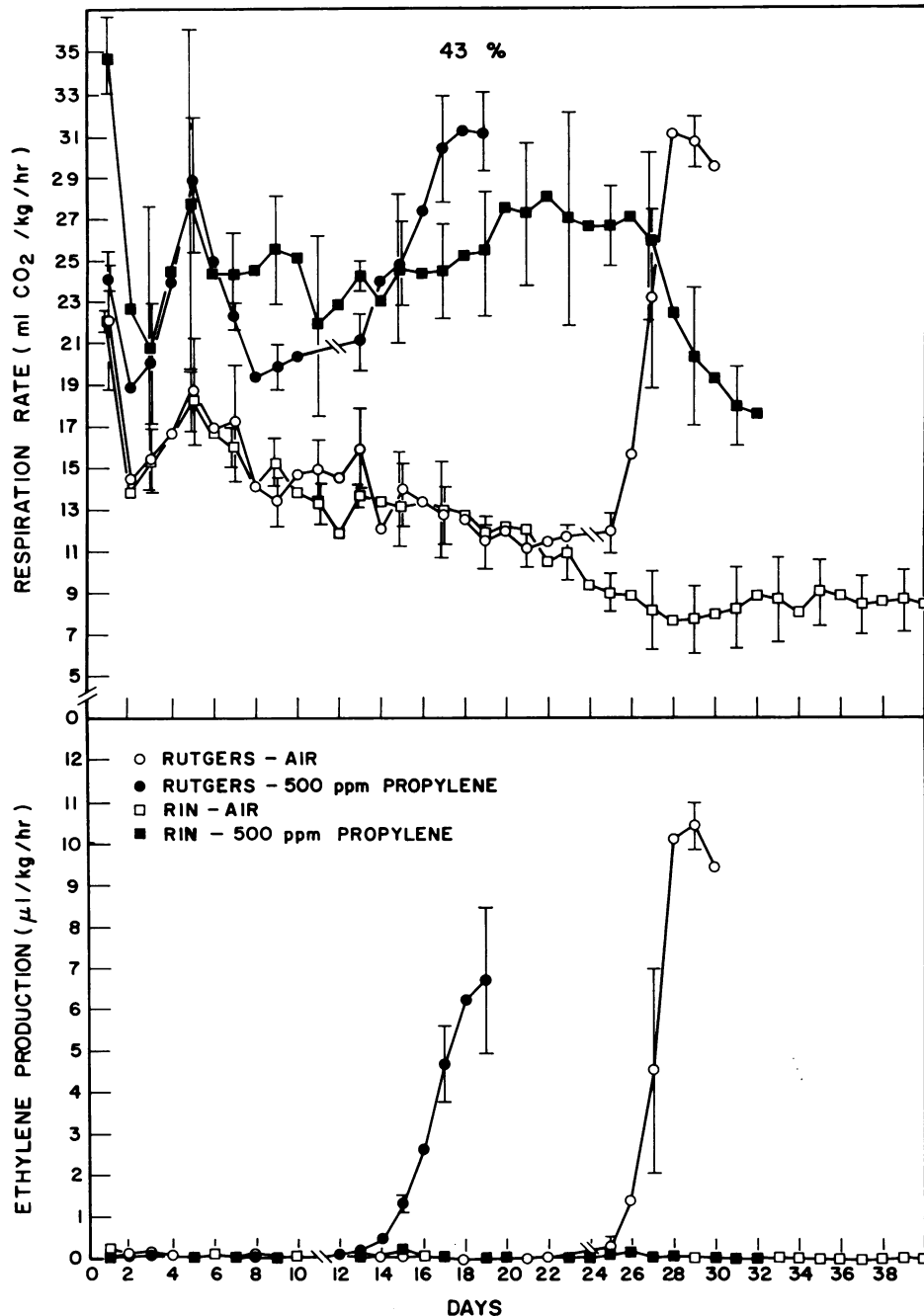


FIG. 2. Average rates of CO₂ and ethylene production by propylene-treated and untreated fruits of Rutgers and *rin*. Other details as for Fig. 1.

ponent of normal ripening once it has been initiated. Rather, it seems that some as yet unidentified and relatively slow change must precede the burst in ethylene production and other concomitant ripening changes. The advanced ripening resulting from treatment with ethylene or propylene could be a result of the acceleration of respiration and of general senescence, a response similar to that found in detached citrus fruits (1, 13). In keeping with these suggestions the lack of characteristic ripening in *rin* fruits, and perhaps in other non-climacteric fruits, may be due to an inability to form a specific cellular component which binds ethylene or its analogues. This specific cellular component is conceived to develop in fruits of normal strains during growth or during aging in detached fruits. The system for the autocatalytic production of ethylene and of other normal components of ripening would

be integrated with the development of this specific cellular component.

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