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Ethylene Production and Respiratory Behavior of the *rin* Tomato Mutant[1]

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ABSTRACT

Little or no change in ethylene or CO_2 production occurred in *rin* tomato mutant fruits monitored for up to 120 days after harvest. Of the abnormally ripening tomatoes investigated, including "Never ripe" (*Nr Y a h, Nr c l_2 r*), "Evergreen" (*gf r*) and "Green Flesh" (*gf*), only *rin* did not show a typical climacteric and ethylene rise.

Fruits from F_1 plants resulting from reciprocal crosses between *rin* and normal plants apeared to ripen normally, but when compared to normal fruit, their ripening was delayed as measured by ethylene and CO_2 production and color change. These fruits produced only one-third to one-half as much ethylene at the peak of production compared to normal fruits.

Exogenous ethylene or propylene treatment did not stimulate ethylene production by *rin* fruits but did stimulate CO_2 production. The CO_2 stimulation persisted only in the presence of the exogenous olefins. Stimulation of CO_2 production could be repeated several times in the same fruit. Wounding stress stimulated both ethylene and CO_2 production in *rin* fruits. It was concluded that *rin* tomato fruits behave like nonclimacteric fruits.

Biale (4) has classified fleshy fruits into two general categories, climacteric and nonclimacteric, depending upon the changes in respiration which occur during ripening and the response to exogenous ethylene. In nonclimacteric fruits, changes in color and composition are not accompanied by a rise in ethylene or CO_2 production (4, 5). Exogenous ethylene causes a rise in respiration when it is applied to the fruits; after it is removed, the respiration rate returns to normal (9). This stimulation of respiration can be repeated in the same fruits several times (4, 23), and a stimulation can occur in fruits at any stage of maturity (3). Vegetative tissues, such as potato tubers, respond in a similar manner (12, 23). Recently, it was shown that propylene (C_3H_6) causes a rise in respiration rate in nonclimacteric fruits with no change in ethylene production (19).

In contrast, a large increase in respiration and ethylene production accompanies ripening in climacteric fruit (4, 23). Exogenous ethylene stimulates respiration and ripening of mature unripe fruits (4). Once stimulated by exogenous ethylene or by their own ethylene, climacteric fruits produce ethylene autocatalytically (6, 19). Propylene treatment of these fruits stimulates both ethylene production and respiration (19).

In climacteric fruits, ethylene is generally considered to be the trigger of the ripening syndrome (21), and its presence at proper levels has been shown to be necessary for normal ripening (7, 16).

Normal tomato fruits have been shown to be of the climacteric type (2, 8, 10, 27, 28), and the response of tomato fruits to ethylene treatment has been investigated (15, 22). As in other tissues (1, 17, 18, 26), tomato fruits respond to wounding stress by greatly increased ethylene and CO_2 production (20).

Reports concerning abnormally ripening tomato mutants have been published (13, 14, 24). Of special interest is the *rin* tomato mutant described by Robinson and Tomes (25) as a spontaneous mutation in a breeding line. Fruits of the *rin* mutant remain green while normal fruits ripen and turn red. The mutant eventually turns a lemon color with little or no lycopene development. Genetic analysis showed that *rin* was a monogenic recessive determined characteristic with no linkage associations except large sepal size. Little work has been done on the physiology or biochemistry of these ripening mutants (11). The respiratory and ethylene production behavior of the *rin* tomato mutant is described in this report.

MATERIALS AND METHODS

Plants of the F_5 generation of *rin* and the normal breeding line (61-37, Fireball × Cornell 54-149) from which it originated were grown in the greenhouse (15 C night, 20 C day) and trained to a single stem. Flowers were tagged at anthesis with only one flower (1st or 2nd) per cluster being pollinated. All others in a cluster were excised. Fruit load per plant was limited to 8 to 10 fruits. Fruits were harvested 30 days after anthesis, the stems were removed carefully, and fruits were washed in water and dried with paper towels. Fruits of similar size were used for all treatments. Fruits from F_1 plants (reciprocal crosses of normal (nor) and *rin* types) and other mutants including "Never ripe" (*Nr Y a h, Nr c l_2 r*), "Evergreen" (*gf r*) and "Green Flesh" (*gf*) were handled in the same manner.

Individual fruits were weighed and placed in pint canning jars with a continuous air flow of approximately 10 ml/min through the chambers. The fruits were held at 20 C under fluorescent lights (16 hr day, 8 hr night). Gas samples from the effluent air stream were analyzed daily for ethylene and CO_2 by gas chromatography. Internal concentrations of ethylene were determined by withdrawing a gas sample from fruits with a syringe while the tomatoes were held under water.

For exogenous ethylene treatments, a gas cylinder was prepared using a mixture of ethylene and compressed air to give

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a concentration of approximately 10 μl/l. Fruits were held in containers with a continuous air flow of 10 ml/min for 2 to 3 days, then gassed with 10 μl/l ethylene for 2 days at the same flow rate and subsequently returned to the air supply. Carbon dioxide production was measured continuously during exogenous ethylene treatment, but ethylene measurements were taken only before and after the period when fruits were being treated. Ethylene and CO_2 production of control fruits was measured continuously for the duration of the experiment.

A static system was used for propylene treatments with three to four tomatoes per 10-liter desiccator. Oxygen was supplied by a Mariotte bottle apparatus, and CO_2 was absorbed by 10 ml of 2 N KOH in a small container in each desiccator. Propylene was added to each desiccator to achieve a concentration of approximately 1000 μl/l. Ethylene production was determined daily by removing 1 ml of gas and analyzing it by gas chromatography. Carbon dioxide production was determined daily by titrating the KOH with 1 N HCl. After CO_2 and ethylene production was determined, the chambers were flushed for 1 min with air, sealed and propylene added again. This was repeated for 4 days.

In the wounding experiments, fruits were placed in containers, and CO_2 and ethylene measurements were taken for 2 to 3 days to obtain initial production rates. Fruits were then cut in half, dipped in 0.5% sodium hypochlorite, rinsed with sterile distilled water, and then returned to the containers with a continuous air flow. Whole control fruits were dipped in sodium hypochlorite and sterile distilled water. Most experiments were repeated three or four times, and each treatment consisted of three to four tomatoes.

RESULTS

Ethylene production of normal fruits began to increase soon after harvest, while *rin* fruits maintained a low and constant production rate during the duration of the experiment (Fig. 1). No surge of ethylene or CO_2 was detected in *rin* fruits which had been monitored up to 120 days from harvest, even though a gradual softening and yellowing of the tissue occurred during this period (data not shown). Fruits from F_1 plants (reciprocal crosses between normal and *rin*) produced much less ethylene than normal fruits, even though color development and softening appeared normal (Fig. 1). The F_1 fruits were delayed in ripening compared to normal fruit as measured by ethylene and CO_2 production and by color change. In many, but not all experiments, the ripening of nor ♀ × *rin* ♂ fruits was delayed less than *rin* ♀ × nor ♂ fruits. Color development in normal and F_1 fruits was first evident 1 or 2 days after the beginning of the rise in ethylene production. *Rin* fruits gradually turned a lemon yellow color after 3 to 4 months.

Internal ethylene concentrations of normal fruits increased rapidly during ripening, but that of *rin* fruits remained low and constant in fruits of varying ages and with differing amounts of yellow color (Table I).

Other tomatoes with abnormal ripening, including "Never ripe" with two genetic backgrounds (*Nr Y a h, Nr c l_2 r*), "Evergreen" (*gf r*) and "Green Flesh" (*gf*) all produced a surge of ethylene (Fig. 2) and had a respiratory climacteric (data not shown) during the time of color change, even though the color change was slow or irregular.

Exogenous ethylene (10 μl/l for 2 days) stimulated endogenous ethylene and CO_2 production and caused earlier ripening of normal fruits (Fig. 3). Exogenous ethylene treatment of *rin* fruits did not stimulate endogenous ethylene production but did cause a stimulation of CO_2 production (but only as long as ethylene was present). Removal of the exogenous ethylene resulted in a decrease in CO_2 production to

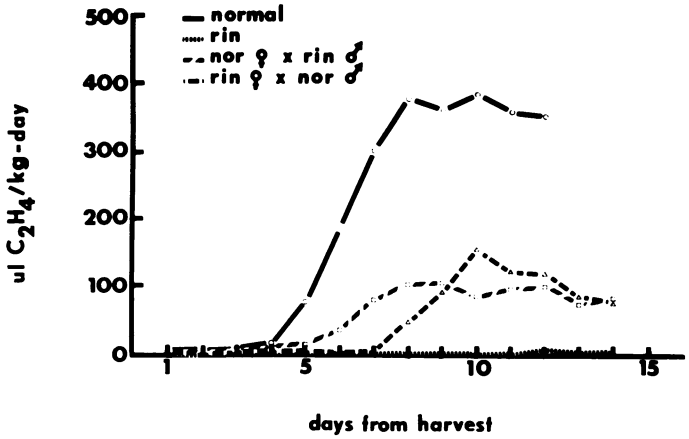

FIG. 1. Ethylene production of fruits of normal, *rin*, normal ♀ × *rin* ♂ and *rin* ♀ × normal ♂ plants. Fruits were harvested 30 days after pollination and held at 20 C.

Table I. *Internal Ethylene Concentrations of Normal and rin Tomato Fruits at Designated Stages of Color Development*

Fruits were harvested 30 days from anthesis and held at 20 C. Each datum is the average of four measurements and is followed by its standard error.

Tomato Fruit	Time from Harvest	Internal C_2H_4 Concn
	days	*μl/l*
Normal		
Green	4	0.18 ± 0.03
Breaker	6	2.15 ± 0.76
Orange	8	11.80 ± 2.71
Red-orange	10	14.70 ± 0.81
Red	18	17.92 ± 2.81
rin		
Green	4	0.29 ± 0.01
Green-yellow	30	0.15 ± 0.04
Yellow-green	63	0.16 ± 0.06
Yellow	102	0.22 ± 0.05

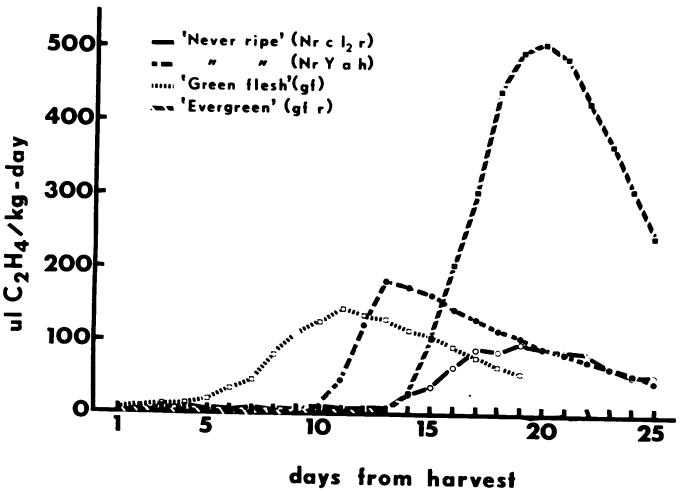

FIG. 2. Ethylene production of fruits of 'Never ripe' (*Nr Y a h, Nr c l_2 r*), 'Evergreen' (*gf r*) and 'Green Flesh' (*gf*) plants. Fruits were harvested 30 days after pollination and held at 20 C.

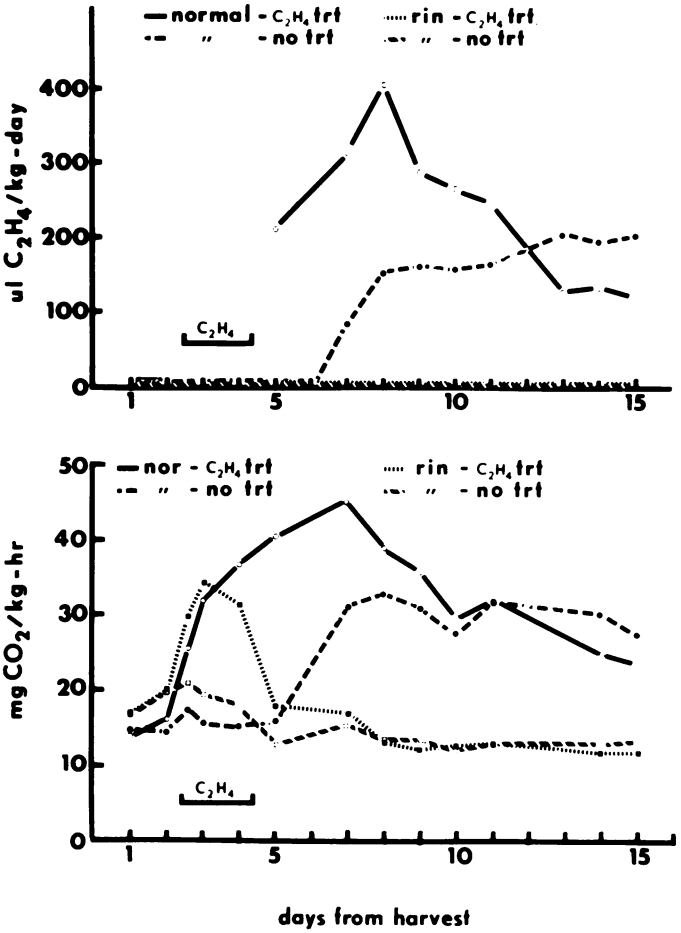

FIG. 3. Ethylene (upper graph) and CO_2 production (lower graph) of normal and *rin* fruits treated with 10 μl/l C_2H_4 for 2 days compared to untreated fruit. Fruits were harvested 30 days after pollination and held at 20 C.

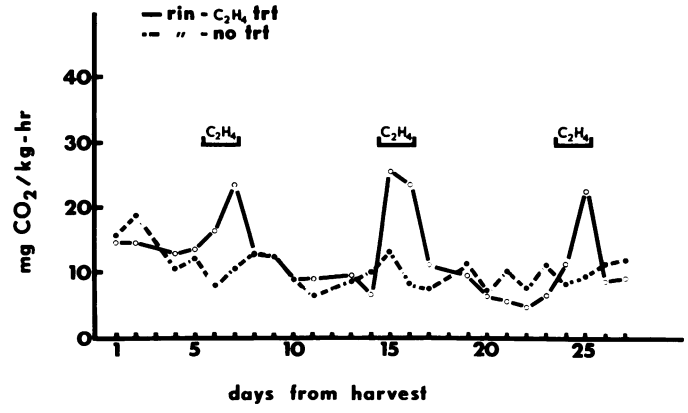

FIG. 4. CO_2 production of *rin* fruits treated three times with 10 μl/l C_2H_4 compared to untreated fruit. Fruits were harvested 30 days after pollination and held at 20 C.

that of the untreated controls. Stimulation of CO_2 production by ethylene was repeated several times with the same *rin* fruits (Fig. 4), and each time the exogenous ethylene was removed the CO_2 production decreased to that of the untreated controls.

The response of normal and *rin* fruits to propylene was similar to the response to ethylene (Fig. 5). Both ethylene and CO_2 production were stimulated by propylene in normal fruit, but only CO_2 production was stimulated in *rin* fruits. The high initial CO_2 levels were probably due to harvesting injury.

To investigate the effect of stress, fruits were cut in half and ethylene and CO_2 production monitored (Fig. 6). Both *rin* and normal fruit exhibited a capacity for wound-induced ethylene production. Increased ethylene and CO_2 production could be detected as early as 6 hr after cutting, and afterwards both declined.

DISCUSSION

Evidence presented for classifying the *rin* tomato mutant as a nonclimacteric fruit includes: (*a*) its lack of a respiratory climacteric and of a rise in ethylene production for up to 120 days from harvest, (*b*) the response to exogenous ethylene which resulted in enhanced respiratory activity only while ethylene was present, (*c*) the repeated stimulation of CO_2 production by repeated ethylene treatments, and (*d*) its response to propylene where CO_2 production was stimulated but ethylene production was not. All of these responses have been shown to be typical of nonclimacteric fruits (3–5, 9, 19, 23). Apparently the *rin* tomato mutant lacks the genetic capacity for autocatalytic production of ethylene or, in terms of McMurchie *et al.* (19), lacks system 2 of ethylene production as do other nonclimacteric fruits. Of the abnormally ripening

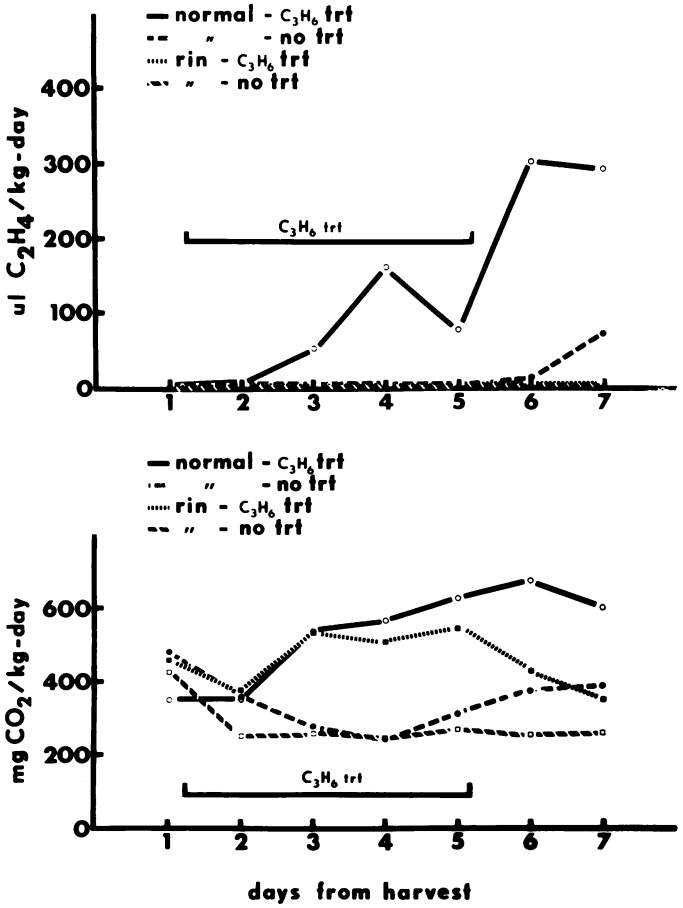

FIG. 5. Ethylene (upper graph) and CO_2 production (lower graph) of normal and *rin* fruits treated with 1000 μl/l propylene for 4 days compared to untreated fruits. Fruits were mature green fruit of undetermined age. Fruits after harvesting were held at 20 C.

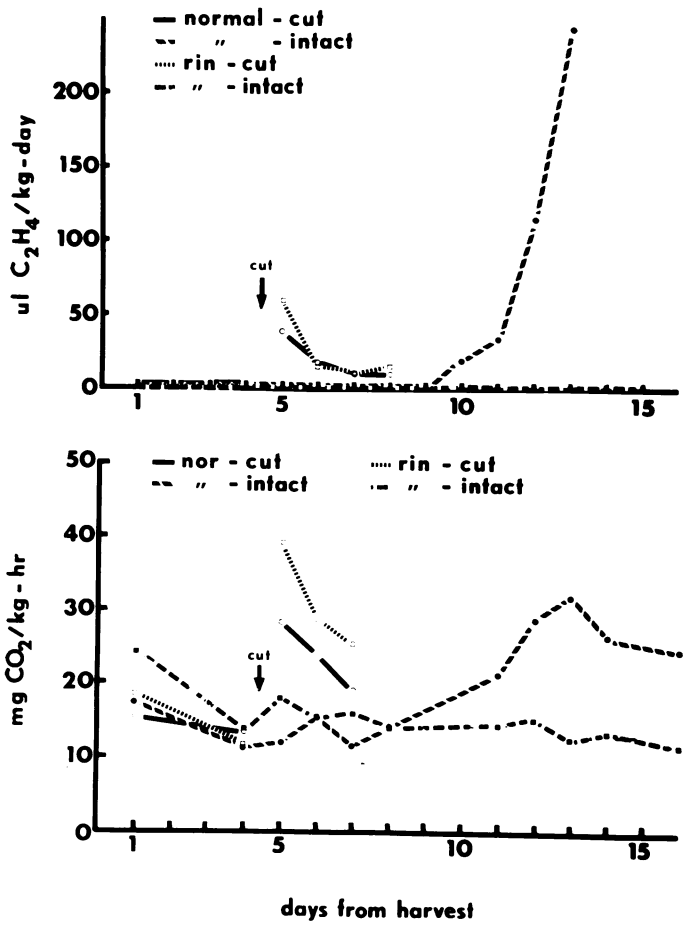

FIG. 6. Effect of wounding stress (cutting of fruits in half) on ethylene (upper graph) and CO_2 (lower graph) production. Fruits were harvested 30 days after pollination and held at 20 C.

tomato mutants investigated, only *rin* fruits showed this peculiar respiratory and ethylene production behavior.

In some experiments there was a clear difference in ripening time between fruits from plants of reciprocal crosses between normal and *rin* types. In all experiments, the rise in ethylene and CO_2 production were consistently delayed in the fruits from F_1 plants compared to normal fruits. In several instances, nor ♀ × *rin* ♂ fruits ripened before *rin* ♀ × nor ♂ fruits. This may indicate that cytoplasmic factors affect the ethylene production mechanism. Exceptions were noted and may have been due to the fact that fruit load per plant was not strictly regulated, and this may have affected ripening behavior (15). Fruits from the F_1 crosses were stimulated to ripen by exogenous ethylene but did not respond as rapidly as normal fruits (data not shown).

The fact that *rin* fruits produced ethylene in response to wounding by cutting suggests that either: (*a*) this stress ethylene was not produced through the same pathway as the ethylene during the climacteric of normal fruit, or (*b*) cutting or wounding stimulated the synthesis of ethylene through the normal pathway, but exogenous ethylene was unable to do so, for undetermined reasons. Abeles and Abeles (1) have suggested that wound or stress ethylene does come from the same pathway from methionine as that produced during the ripening of normal fruit. They also pointed out that the efficiency of conversion of labeled methionine to ethylene fell 50% after wounding which might indicate another pathway for at least part of the stress-induced ethylene. There is also the possibility that wounding or stress merely stimulates system 1 production of ethylene but is incapable of stimulating system 2 in nonclimacteric fruits (19). *Rin* fruits were also observed to produce ethylene in response to fungal invasion, but it was not determined if the ethylene was produced by the organism or the fruits. Fruits from F_1 plants resulting from crosses between *rin* and normal plants also produced large amounts of ethylene in response to wounding.

One of the major differences between climacteric and nonclimacteric fruits is the ability of the fruits to produce ethylene autocatalytically. The *rin* mutant should provide very valuable information concerning this basic difference between climacteric and nonclimacteric fruits, since there is now the possibility of comparing tomato fruits from breeding lines which are very closely related genetically but exhibit a difference in ripening behavior. The *rin* mutant may also serve as a tool for investigations concerning the biosynthetic pathway of ethylene production.

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