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Effect of Endogenously Synthesized and Exogenously Applied Ethanol on Tomato Fruit Ripening[1]

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill. var Castlemart) fruit ripening was inhibited by tissue concentrations of ethanol that were produced by either exposure to exogenous ethanol vapors or synthesis under anaerobic atmospheres. Ethanol was not detected in aerobically ripened tomato fruit. Ripening was not inhibited by exposure to methanol at an equivalent molar concentration to inhibitory concentrations of ethanol, while ripening was slightly more inhibited by *n*-propanol than by equivalent molar concentrations of ethanol. The mottled appearance of a few ripened ethanol-treated fruit was not observed in *n*-propanol-treated fruit.

Higher plant tissues synthesize ethanol and acetaldehyde when exposed to anaerobic conditions or during the ripening of certain fruit (13, 14, 18). Anaerobic ethanol accumulation has been demonstrated in a variety of higher plant tissues and species, including leaves from 50 species (17), fruits (25), roots (2), and seeds (3, 8). Other stresses, such as air pollution and water deficit, may also induce ethanol biosynthesis (16). The typical occurrence of very low ethanol content in unstressed vegetative organs (2, 12, 17, 21) may account for the relatively few studies of the effects of ethanol on plant metabolism.

Under nonstress conditions, exogenously applied ethanol has been reported to have a number of effects, including both promotion and inhibition of growth of a wide range of species (12), increased respiration of carrot root discs (19), and delayed senescence of oat leaves (27) and carnation flowers (9). In the latter case, inhibition of senescence was accompanied by inhibition of ethylene synthesis. Several recent studies suggest the possibility that ethanol accumulation may account for the injury sustained to plant tissues during anoxia (1, 8, 12). This hypothesis has been rejected by Jackson *et al.* (12) as their experimental results and their literature survey indicated that plants are resistant to concentrations of ethanol likely to accumulate during anaerobiosis.

In contrast to vegetative plant organs, ethanol biosynthesis is a normal occurrence in the development of some fruits (13, 28). For example, ethanol and acetaldehyde increase dramatically during ripening of pears (14). The metabolic effects of ethanol on fruits are of particular interest not only because ethanol is an endogenous metabolite but also because an increasing number of fruits are stored in low oxygen atmospheres. In addition, the products of anaerobiosis can affect ripening and fruit sensory quality. Postharvest ethanol treatments reduced the astringency and, at some stages of maturity, stimulated respiration and ethylene synthesis of Japanese persimmon (10, 11). Acetaldehyde promoted ripening and ethylene synthesis in pears (14) and promoted color formation and a climacteric-like respiratory rise in blueberry and strawberry fruit (15). Treatment of blueberries, tomatoes, and pears with ethanol or acetaldehyde vapors increased their sugar content and sugar-acid ratio and induced desirable flavor changes (23). These compounds were less effective in promoting color changes than ethylene but had a more positive effect on flavor.

A previous communication from this laboratory (26) reported inhibition of ripening of tomato fruit by ethanol. In the study described here, we have further investigated this phenomenon to better characterize the role of ethanol in fruit ripening.

MATERIALS AND METHODS

Plant Material. Tomatoes (*Lycopersicon esculentum* Mill. var Castlemart) were grown in the field according to normal cultural practices. Fruit were harvested at the mature-green stage and were either used the same day or stored at 12°C for not more than 48 h. Unblemished, uniformly shaped fruit with a mean fresh weight of about 170 g were washed in a 1% sodium hypochlorite solution (20% household bleach), rinsed, and air dried. Treatments were blocked with respect to size and color variation. All experiments were performed at 25°C.

Treatment with Gasses and Vapors. Twelve fruit (about 2 kg fresh weight) were placed in a 20-L jar. Beakers containing 12 g KOH were placed in the jars to trap evolved CO_2 and maintained the CO_2 concentration below 0.5%, as confirmed by periodic analysis.

Fruit were exposed to alcohol vapors by applying alcohol to a 6 × 35 cm strip of Whatman No. 1 filter paper taped at one end to the rim of the jar. The other end of the paper strip rested on the bottom of a beaker in the jar. Reagent grade 100% methanol, ethanol, or *n*-propanol was pipetted along the length of the filter paper strip. Excess alcohol collected in the beaker and did not come into contact with the fruit. The jars were sealed with rubber stoppers and left for 24 h.

When 4 mL of ethanol was added to an empty 20-L jar, all the liquid evaporated and the final ethanol concentration in the gas phase was 10.5 ± 0.5% (Fig. 1A). Apparently, this is close to saturation at 25°C, as 8 mL of ethanol did not completely evaporate, and the final concentration was still around 10.5% (Fig. 1B). However, when KOH was added to absorb CO_2, both 4 mL and 8 mL of ethanol completely evaporated and the concentration of ethanol vapor did not exceed 5% before declining to low levels (Fig. 1, A and B). Thus, KOH appeared to absorb a large proportion of the ethanol. This was further demonstrated by the discoloration of the KOH. When fruit and KOH were both present, the ethanol vapor concentration did not exceed 4% initially and fell to very low levels, essentially to zero, when 4 mL ethanol were added (Fig. 1A). This implies that the

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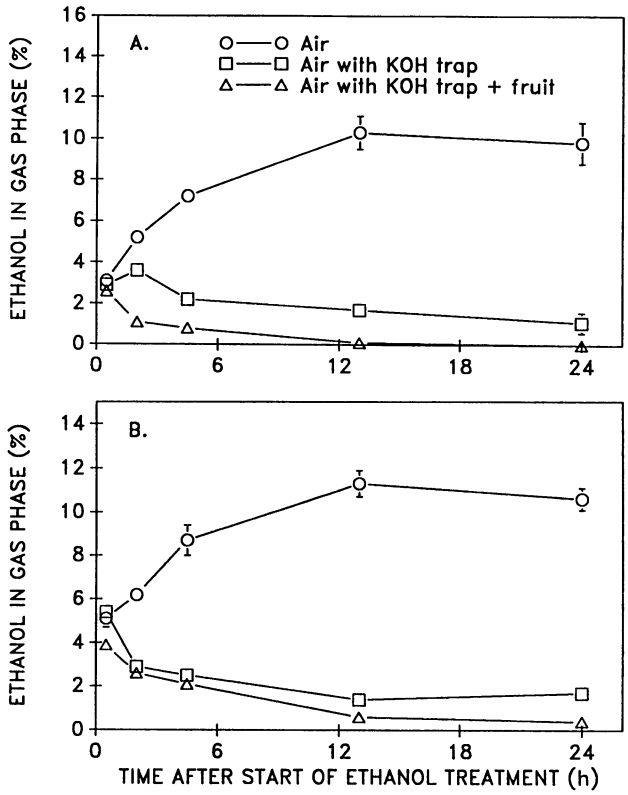

FIG. 1. Ethanol concentration in the gas phase of 20-L glass jars vs. time following addition of (A) 4 mL or (B) 8 mL of ethanol in the presence or absence of a CO_2 trap (KOH) and fruit. Data represent triplicate samplings. Error bars represent standard error of the mean where this value exceeds the width of the symbols.

fruit and KOH competed for ethanol vapor. Both the kinetics of ethanol vaporization and the extent of KOH discoloration suggest that the KOH absorbed much less ethanol when the fruit were present than when the fruit were absent.

Fruit were exposed to humidified N_2 gas or a mixture of humidified air ± ethylene in a flow-through system at 10 L h^{-1}. An anaerobic environment was confirmed by sampling the gas phase for oxygen by gas chromatography. The ethylene concentration was uniform between treatments in a single experiment but varied between experiments from 12 to 18 μL L^{-1}.

Ripening Assay. Ripeness was subjectively assayed at about the same time daily, while the fruit were continuously exposed to ethylene in air. Fruit were briefly removed from the jars and visually scored for ripeness using a scale of zero to six in which zero represents mature-green and six represents red-ripe. (Scale compiled and published by Tomato Division, United Fresh Fruit and Vegetable Assn.)

Ethanol Measurement. Ethanol vapor in the gas phase of the jars was measured by flame ionization gas chromatography using a 3 m × 3 mm column packed with 5% Carbowax on Chromosorb G and maintained at 100°C. Peak heights were compared to those of a prepared standard. The calculated ethanol concentration in the gas phase of our prepared standards agreed closely with that measured with Kitagawa Precision Gas 104SA ethanol detector tubes (Matheson Gas Products).

Tissue ethanol content was assayed in wedges of fruit. Seven g of tissue were homogenized for 1 min in 21 mL of 1.8% (w/v) $Ba(OH)_2$ in a tissue homogenizer at high speed. Twenty-one mL of 2.0% (w/v) $ZnSO_4$ were added, and the mixture vigorously shaken before centrifugation in a clinical centrifuge at high speed for 5 min. Aliquots of the cleared supernatant were added to a cuvette with 1.0 mL of a reaction mixture containing 1.3 mg NAD and 12.5 units alcohol dehydrogenase in 0.5 M potassium phosphate (pH 9.0). Reduction of NAD was measured by changes in the absorbance of NADH at 304 nm and was correlated to the ethanol content in the supernatant by a standard curve. The recovery of ethanol injected into tomato fruit slices was consistently greater than 90%, and the extract did not significantly interfere with the assay.

Measurement of Respiratory Rates. Two tomato fruit were placed daily in 4-L glass jars that were sealed with a rubber stopper. The jars were rotated after 1 h and the CO_2 concentration in a 1-mL sample of the headspace was measured by gas chromatography.

RESULTS

Kinetics of ethanol absorption by treated fruit was determined by measuring the ethanol content of fruit wedges over time. When fruit were treated with 4 mL ethanol in 20-L jars, the maximum ethanol concentration of 1.4 mg g^{-1} fresh weight was reached about 12 h after treatment (Fig. 2). Thereafter, the ethanol content of the tissue declined. Because of the presence of the KOH, it cannot be determined if the loss of ethanol from the tissue was due to metabolism or absorption by the KOH. The ethanol content of fruit treated with 8 mL ethanol increased over the 24 h treatment and reached about 2.5 mg g^{-1} fresh

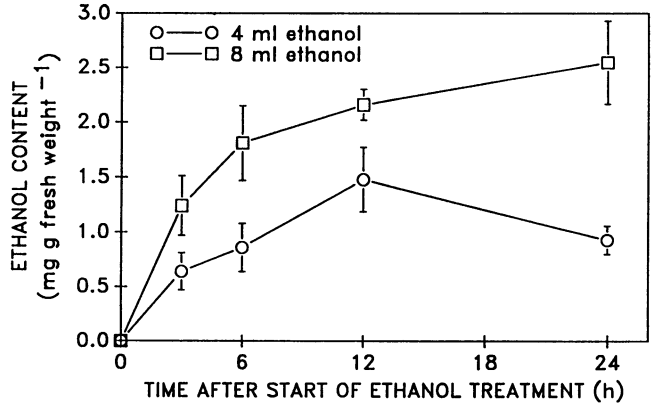

FIG. 2. Ethanol content of tomato fruit *versus* time after exposure to 4 or 8 mL of ethanol in 20-L glass jars. Data represent the mean of three or four fruit. Error bars represent standard error of the mean. A representative experiment is presented.

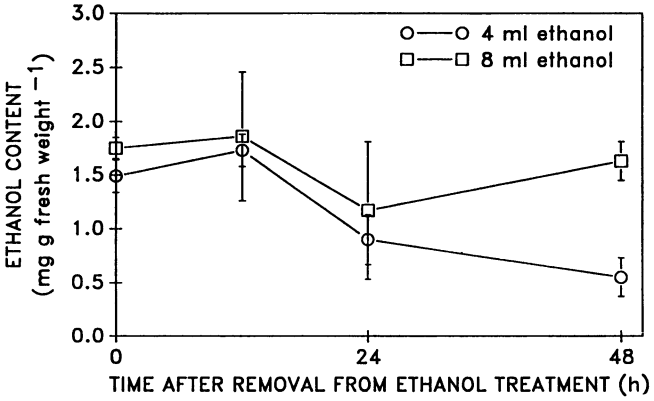

FIG. 3. Ethanol content of tomato fruit *versus* time after exposure to 4 or 8 mL of ethanol in 20-L glass jars for 12 h. Data represent the mean of three fruit. Error bars represent standard error of the mean. A representative experiment is presented.

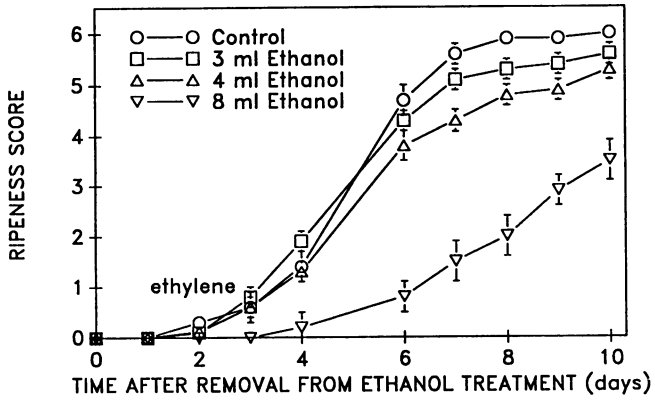

FIG. 4. Ripening of tomato fruit after treatment with ethanol for 24 h. Ethylene gas was continuously supplied after removal of the fruit from the ethanol treatments. Ripeness was qualitatively assessed daily. Data represent the mean of 12 fruit. Error bars represent standard error of the mean. A representative experiment is presented.

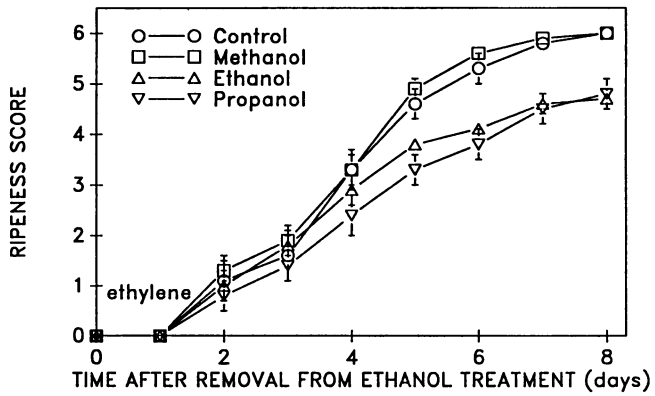

FIG. 5. Ripening of tomato fruit after treatment with methanol, ethanol, or *n*-propanol for 24 h. Amounts of alcohols equivalent on a molar basis to 4 mL ethanol were added to 20-L glass jars. Ethylene gas was continuously supplied after removal of the fruit from the ethanol treatments. Data represent the mean of 12 fruit. Error bars represent standard error of the mean. A representative experiment is presented.

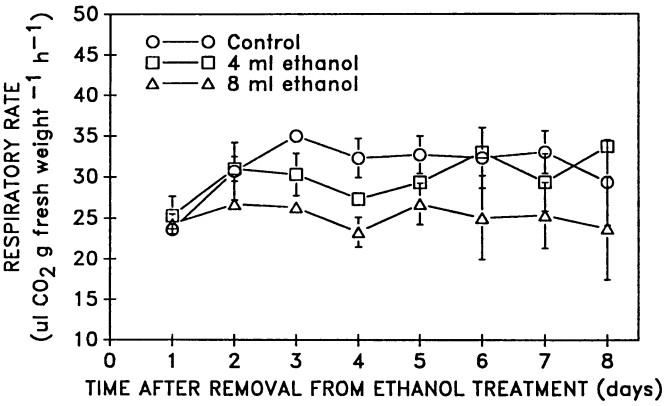

FIG. 6. Respiration of tomato fruit after treatment with 4 or 8 mL ethanol for 24 h. Ethylene gas was continuously supplied after removal of the fruit from the ethanol treatments. Data represent the mean of three replicates with two fruit each. Error bars represent standard error of the mean.

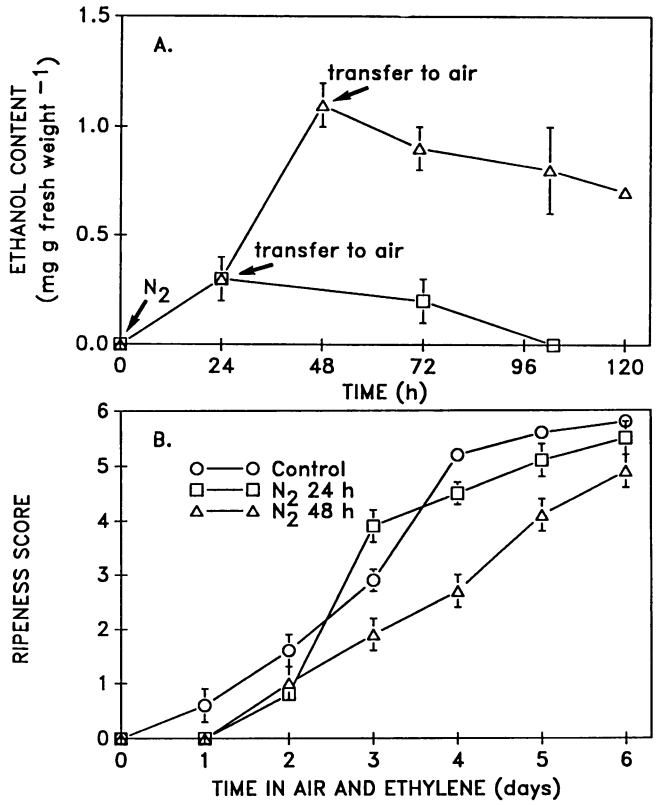

FIG. 7. (A) The ethanol content of tomato fruit after 24 or 48 h in nitrogen and after removal from the anaerobic environment. Data represent the mean of three or four fruit. Error bars represent standard error of the mean. (B) Ripening of tomato fruit after exposure to an anaerobic environment. Data represent the mean of 12 fruit. Error bars represent standard error of the mean. Representative experiments are presented.

weight (Fig. 2). In contrast to the gradual decline in ethanol content over 48 h in fruit treated with 4 mL of ethanol, the ethanol content of fruit treated with 8 mL ethanol remained relatively constant during this period (Fig. 3). For both treatments, 80–90% of the ethanol added to the jars was detected in the fruit. Ethanol was not detected in untreated tomato fruit at several early stages of ripening under aerobic conditions (data not shown).

Fruit were treated with methanol, ethanol, or *n*-propanol for 24 h, and then continuously with ethylene gas to assess the effects of alcohols on ripening. Treatment of the fruit with 8 mL ethanol markedly inhibited ripening, delaying its initiation by several days as well as slowing its rate (Fig. 4). Ripening was also retarded by 3 mL and 4 mL ethanol. Some of the ethanol-treated fruit ripened nonuniformly; ripening was fully inhibited in some sections of the fruit while other sections ripened normally, resulting in a mottled appearance. Most of the fruit treated with ethanol eventually reached the red-ripe stage, although a few of the fruit treated with 8 mL ethanol developed surface pitting. Ripening of fruit treated with 2.8 mL methanol (the molar equivalent to 4 mL ethanol) was not significantly different from that of untreated fruit (Fig. 5), nor was ripening inhibited by concentrations of methanol equivalent to 5.2 mL ethanol (data not shown). Ripening was slightly more inhibited by a molar concentration of *n*-propanol equivalent to 4 mL of ethanol than by ethanol (Fig. 5). The *n*-propanol-treated fruit also exhibited less damage and did not produce the mottling observed on a few ethanol-treated fruit.

The typical climacteric burst of CO_2 production during tomato fruit ripening was detected in control fruit and fruit exposed to

4 mL ethanol (Fig. 6). The respiratory rate was similar to control fruit during d 1 and 2 in fruit exposed to 4 mL ethanol. During d 3 and 4, it remained relatively constant and less than the control. On d 5, the respiration rate started to increase to a level reached on d 6 that was similar to control fruit. In contrast, there was no significant change from a nearly constant respiration rate throughout the experiment for fruit exposed to 8 mL ethanol.

Anaerobic conditions stimulated ethanol content (presumably synthesis) of mature-green tomato fruit (Fig. 7A). Tissue ethanol concentrations of 0.3 mg g^{-1} fresh weight and 1.2 mg g^{-1} fresh weight were detected after 24 and 48 h of anaerobiosis, respectively. Thus, a similar ethanol tissue concentration was reached either after 48 h in N_2 or after 24 h of exposure to 4 mL ethanol (Fig. 2). The ethanol content of fruit in a N_2 atmosphere declined slowly for 72 h upon return to air (Fig. 7A). Anaerobic conditions for 48 h inhibited both the initiation and rate of ripening (Fig. 7B). This inhibition was similar in magnitude to the inhibition observed with 4 mL ethanol in 20-L for 24 h (Figs. 4 and 7B).

DISCUSSION

The failure to detect ethanol in ripening tomato fruit under aerobic conditions in this study suggests that tomato fruit do not synthesize ethanol as part of their normal ripening, as do oranges (7), pears (14), and strawberries (20). Therefore, the ethanol detected in tissue of ethanol-treated tomato fruit most likely resulted from absorption of ethanol vapors rather than from the synthesis of ethanol by the fruit. The partition coefficient for ethanol between gas and water phases dictates that essentially all of the ethanol would have been absorbed by the 2 kg of tomato fruit in the 20-L jars (17). This conclusion is supported also by our finding that the rate of disappearance of ethanol from the gas phase was similar whether tomato fruit or water-saturated filter paper represented the water phase (data not shown). It would seem, then, that the critical factor in treatment of tissue with ethanol vapors is the absolute amount of ethanol added, rather than the gas phase concentration.

In general, ethanol was lost by tomato fruit very slowly or not at all, whether the ethanol was exogenously supplied (Fig. 3) or synthesized by the tissue (Fig. 7A). The loss of ethanol from the fruit was much slower than from pea cotyledons (3), which have been shown to have the capacity to metabolize ethanol (4, 5). Tomato fruit, then, may not have the capacity for rapid ethanol metabolism. Although plant tissues are capable of ethanol metabolism (6), it has not been detected in all cases (19, 24, 29). Ethanol may be slowly lost from tissue by evaporation (3).

Ethanol and propanol clearly inhibited tomato fruit ripening in the presence of ethylene in this study. A specific effect of these alcohols seems likely, since methanol had no effect on ripening (Fig. 5). The concentration of ethanol in fruit exposed to ethanol vapors should not have been toxic in our experiments (12). Clearly, the 3 and 4 mL treatments were not toxic since fruit eventually ripened without visible injury. The respiratory rate of tomato fruit exposed to 4 mL ethanol was significantly reduced, and the climacteric rise did not occur in these fruit until 3 d after it occurred in control fruit (Fig. 6). Doubling the ethanol concentration to 8 mL prevented the climacteric rise in respiration, but neither stimulated nor depressed the respiration rate compared to its initial value. Thus, it appears that the alcohols did not act as general toxins or metabolic inhibitors since they did not depress respiration below its initial value nor stimulate a wound response.

A possible explanation for the inhibition of tomato fruit ripening by ethanol is the inhibition of ethylene action. In support of this hypothesis, in preliminary studies we have observed inhibition of ethylene-induced tomato petiole epinasty by ethanol vapors (MO Kelly, ME Saltveit, unpublished data). Ethanol may also regulate ethylene synthesis (9–11, 15, 26), but any such effects could not have been a factor in these experiments since optimal concentrations of ethylene were added to induce ripening.

Since ethanol is often used to surface-sterilize tissue during preparation for physiological studies, care must be exercised that residual ethanol does not adversely affect the experiment. For example, the exceptionally long time it took discs of mature-green tomato fruit to ripen in a recent publication (22) could have resulted from a 60 sec immersion in 80% ethanol used to sterilize the tissue after excision.

Concentrations of ethanol accumulated in tomato fruit tissue either under anaerobic conditions or during exposure to ethanol vapor which were inhibitory to fruit ripening. Ethanol may, therefore, be an important regulator of fruit development, both in species in which it is synthesized under normal ripening and in fruits held in controlled atmosphere storage.

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