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# Effect of Alcohols and Their Interaction with Ethylene on the Ripening of Epidermal Pericarp Discs of Tomato Fruit[1]

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## ABSTRACT

**Ethanol concentrations that were induced in pericarp discs of mature-green tomato fruit (*Lycopersicon esculentum* Mill, cv Castlemart) either by anaerobic metabolism or by exposure to ethanol vapor inhibited ripening without increasing the rate of ion leakage. Inhibition of ripening (*i.e.* lycopene synthesis) of excised tomato pericarp tissue by ethanol vapor was reversed by increasing concentrations of the plant hormone ethylene. A Lineweaver-Burk plot indicated noncompetitive interaction between ethanol and ethylene. Methanol and *n*-propanol also inhibited lycopene synthesis without significantly increasing ion leakage. The similar inhibitory effects of methanol, ethanol, and *n*-propanol at concentrations which did not stimulate ion leakage, and the relationship between activity and lipophilia of the alcohols suggest that their mode of action was through disruption of membranes associated with ethylene action.**

Ethanol production and accumulation by higher plants is more common than previously thought (19, 20). Anaerobic metabolism and ethanol production generally result from low $O_2$ concentrations which can be caused by reduced external $O_2$ levels (8, 14), increased resistance to $O_2$ diffusion (1, 8), and high respiration rates in tissue with low surface area to volume ratios (2). Air pollutants (18, 19), freezing (18), ice encasement (1), mechanical injury (18), and drought (18) are stresses which can stimulate ethanol production. The concomitant increase in $CO_2$ concentration with $O_2$ consumption in impermeable tissue stimulates anaerobic metabolism (5). These many factors can lead to ethanol accumulation in aerobically germinating seeds (8), vascular tissue of large woody plants (20), and ripening fruit (2, 8, 15). The physiological effects of ethanol produced under these conditions are not known.

It was recently established that ethanol concentrations inhibitory to ripening could be produced in tomato fruit tissue either by a few days of anaerobiosis (17) or by exposure to ethanol vapor (17, 30). Inhibitory ethanol concentrations delayed the increase in ethylene and carbon dioxide production, Chl loss, and lycopene synthesis. Effective ethanol concentrations were similar to those endogenously synthesized in other plants during anoxia (5, 14), stress (18), and fruit ripening (2, 15). However, ethanol was not detected in aerobically ripening whole tomato fruit (17).

Exogenous application of high levels of ethanol can retard the senescence of cut carnation flowers (12, 13, 33), excised oat leaves (31), and inhibit the sprouting of potato tubers (4). However, ethanol did not delay the senescence of cut lily and tulip flowers (13); flowers in which ethylene does not appear to control senescence. In contrast to these inhibitory affects, ethanol treatments increase the quality and stimulate ripening of harvested Japanese persimmons, blueberries, tomatoes, and pears (15, 26). Plants tolerate these high levels of ethanol, which are usually many times the concentration produced during stress. Jackson *et al.* (14) reported that damaging levels of ethanol ranged from 12- to 200-fold higher than the concentrations found in a large number of flooded plants.

Previous communications from this laboratory have shown that ethanol appears to act through both an inhibition of ethylene synthesis and action (30). The activity of the ethylene forming enzyme was reduced and ACC,[2] the immediate precursor of ethylene synthesis, accumulated in ethanol treated tissue. However, the relationship between ethanol inhibition and ethylene stimulation of ripening was not studied. Conversion of ACC to ethylene has also been reported to be inhibited by ethanol in the holding-solution of cut carnation flowers (13). Another problem raised by a previous study was the reported inability of methanol to mimic ethanol inhibition of tomato fruit ripening (17). This observation proved to be inconsistent with other studies showing a progressive effect of a number of organic compounds on various metabolic functions with increasing mol wt (4, 21, 22, 31).

Research reported in this paper clarifies the interaction between ethanol and ethylene, and the effects of other alcohols on tomato fruit ripening.

## MATERIALS AND METHODS

### Preparing Epidermal Pericarp Discs

Mature-green tomato fruit (*Lycopersicon esculentum* Mill., cv Castlemart) were hand-harvested from plants grown at the Vegetable Crops field station in Davis, CA under normal cultural practices. Unblemished, uniformly shaped fruit with a mean fresh weight of around 170 g were washed in a 0.26% sodium hypochlorite solution (1:20 dilution of 5.25% commercial bleach to water), rinsed with sterile deionized water, and air dried in a sterile transfer hood. One-cm epidermal pericarp discs were excised with a cork borer from the equatorial region of the fruit. Adhering locular material was

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[2] Abbreviation: ACC, 1-aminocyclopropane-1-carboxylic acid; FW, fresh weight.

trimmed away to produce 3 mm thick discs; each disc weighed 0.350 ± 0.014 g (±4%) FW. The discs were washed three times in sterile deionized water, blotted dry, and eight discs were placed epidermis down in 15 × 100 mm plastic Petri dishes.

Discs of epidermal pericarp tissue were used in this study because they exhibit all the physiological changes found in ripening whole fruit (29, 30) and offer many advantages over slices and whole tomato fruit. Unlike whole fruit or slices that are composed of many different tissues, pericarp discs are composed of relatively homogenous tissue. Applied solutions and vapors are rapidly absorbed through the cut surfaces and a large number of uniform discs can be prepared from one fruit, thereby reducing variability within an experiment.

Since preliminary experiments showed that light green discs produced less lycopene than dark green discs, discs were blocked by the intensity of their green color among the treatments to allow removal of this source of variability during the statistical analysis of the data. Care was also taken to make sure the discs remained epidermis surface down, since lycopene synthesis was also affected by the orientation of the discs. Dishes were held overnight at 20°C in a flow of humidified, ethylene-free air to allow dissipation of the wound response. Microbial contamination was not a problem in any of the experiments.

### Alcohol Treatments

Two dishes containing four or eight discs each (each disc weighed 0.35 ± 0.01 g FW) were placed in a 4-L glass jar. The bottom portion of a 15 × 60 mm plastic Petri dish containing a folded 7-cm diameter Whatman No. 1 filter paper was placed on top of the two dishes. Reagent grade, absolute methanol, ethanol, or *n*-propanol was pipetted onto the filter paper wick. The jar was immediately capped with a rubber stopper held in place with a large rubber band. The maximum volume of alcohol applied to the filter paper (0.7 mL) was visually determined to have evaporated within 1 h. Most experiments used a 3 h exposure and 0.3 mL of alcohol. All filter papers were dry to the touch at the end of the usual 3 h treatment period. In previous experiments (17), KOH that had been included in the jars to absorb $CO_2$ appeared to also absorb some of the added ethanol. It was not necessary to include a $CO_2$ absorber in these jars since $CO_2$ levels were usually below 0.2% and did not exceed 0.5% in any experiment. At the end of the alcohol treatment phase, the rubber stoppers were removed and the dishes were either transferred to a 20-L jar with a flow of 20-L $h^{-1}$ humidified, ethylene-free air, or ion leakage was determined as described below. In some experiments, the discs were subsequently exposed to ethylene in air mixtures.

Ethanol treatments were also applied as solutions to the locular surface of discs. Twenty μL of 0.0 to 35% (v/v) ethanol solutions were applied to each disc with a 0.1-mL syringe. A 0.45-micron filter between the syringe and the sterile hypodermic needle sterilized the solutions as they were applied. After 3 h the discs had absorbed the solutions and the dishes were placed in a 20-L jar flushed with humidified, ethylene-free air in a room whose temperature was 20°C. Immediate recovery of ethanol added as a solution was around 99%.

### Measuring Ion Leakage

Two discs from each treatment were placed into a tared 100-mL beaker and weighed. Thirty mL of 0.3 M mannitol was added to each beaker, and the beakers were again weighed. In preliminary experiments, conductivity was observed to increase rapidly when both control or treated discs were held in deionized water. However, the variability was large and the statistical significance of the differences among treatments was small. Hypothesizing that deionized water placed too much of an osmotic stress on the discs (34), mannitol solutions were used to reduce the osmotic stress. The rates of ion leakage from control and ethylene treated discs were very consistent and the significant differences between control and treated discs were larger when from 0.2 to 0.4 M mannitol solutions were used (Fig. 1). A 0.3 M mannitol solution was used in all subsequent conductivity measurements.

The beakers were shaken at 2 cycles/s between conductivity readings. At periodic intervals, the shaker was stopped and the conductivity of the solutions was measured with an Ex-Tech model 480 digital conductivity meter. The beakers were then heated to boiling for 5 min, cooled overnight at 0°C with shaking, warmed to room temperature, and made to their initial weight with deionized water. After shaking for 1 h at room temperature, the total conductivity was measured. Rates of ion leakage are expressed as percent of the total conductivity. The uniformity of the pericarp discs resulted in a low coefficient of variability of less than 3.5% among replicates within treatments when two discs were used per replicate.

### Ethylene Treatments

After the alcohol treatments, some dishes were transferred to 4-L glass jars that were flushed at 10 L $h^{-1}$ with humidified air containing 0.0, 0.25, 2.5, or 25 μL/L ethylene. Most discs were ripened at 20°C for 5 d in 20-L glass jars that were flushed with humidified air containing 10 μL/L ethylene.

### Lycopene Assay

Dishes were stored at −20°C until the discs were assayed for lycopene. Two discs from each dish were weighed, ho-

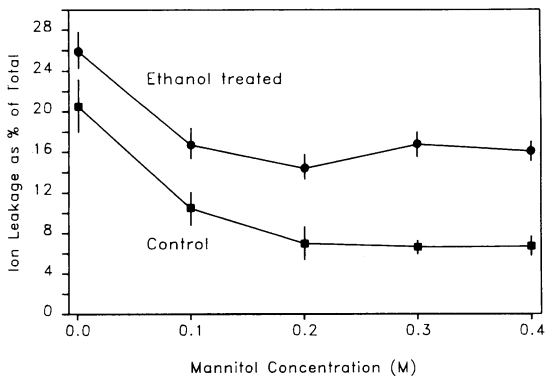


**Figure 1.** Effect of mannitol concentration on the ion leakage, as a percent of the total ions, from pericarp discs exposed to 0.0 or 0.3 mL ethanol for 3 h (see "Materials and Methods"). Error bars represent SE of the mean.

mogenized with 10 mL of acetone for 10 s in a Sorvall Omnimixer at maximum speed, centrifuged at maximum speed in an International clinical model CL table top centrifuge, and the absorbance of the clear supernatant was measured at 503 nm. Lycopene content is expressed as absorbance per gram fresh weight.

### Ethanol Assay

Two discs from each dish were weighed and homogenized for 10 s in 10 mL of 1.8% (w/v) $Ba(OH)_2$ in the Sorvall homogenizer at high speed. Ten mL of 2.0% (w/v) $ZnSO_4$ was added, and the solution homogenized again for 10 s. The mixture was centrifuged for 3 min in the clinical centrifuge at maximum speed. A 30 $\mu$L aliquot of the clear supernatant was 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 buffer (pH 9.0). Reduction of NAD was measured as changes in the absorbance of NADH at 304 nm. The ethanol content in the supernatant was calculated from a standard curve. The recovery of ethanol spiked tomato tissue was consistently greater than 90%, and the extract did not interfere with the assay.

Each experiment contained at least three replicates and was repeated at least twice with similar results.

## RESULTS

All of the liquid ethanol that was applied to the filter paper wicks in the 4-L jars had volatilized and been absorbed by the pericarp discs within 3 h. This rate of volatilization and absorption agrees with previous studies in this laboratory using tomato pericarp discs and slices (30) and whole fruit (17, 30). Recovery of ethanol from the 16 discs exposed to 0.0 to 0.5 mL in a 4-L jar for 3 h (the standard treatment regime) was 96.9 ± 1.9% (Fig. 2, zero time). A linear regression equation fitted to the data had a correlation coefficient ($r$) of 0.996. The equation for the ethanol concentration of the 16 discs (about 5.6 g FW) can be expressed either as mg EtOH $g^{-1}$ FW = 0.08 + 139.76 (mL EtOH added), or as mmoles EtOH $g^{-1}$ FW = 0.002 + 3.034 (mL EtOH added).

Application of 20 $\mu$L of 0.0 to 35% (v/v) ethanolic solutions directly to the locular surface of pericarp discs resulted in a generally linear decrease in lycopene synthesis (Fig. 3). Rates of inhibition were slightly better than those produced by exposing discs to ethanol vapor. However, a visual examination of sectioned discs 6 d after treatment revealed a lens shaped portion of tissue near the epidermis with higher lycopene content than the rest of the discs treated with inhibitory levels of ethanol. A similar lens shaped portion of tissue has also been observed in tomato pericarp discs treated with silver ions, another inhibitor of ethylene action (29). Apparently the ethanolic solutions were not affecting the discs uniformly. This observation along with the higher degree of variability among treatments and between experiments resulted in the use of ethanol vapor exposures in all subsequent experiments.

### Effect of Duration of Exposure and Concentration of Ethanol

Ethanol loss from discs previously exposed to ethanol vapor was fairly rapid upon their transfer to a flow of humidified air (Fig. 2). An exponential curve fitted to the ethanol decay data produced the equation: ln mg[EtOH] = ln original mg[EtOH] - (0.0664 × h), with an $r$ of 0.98. The half-time for ethanol decay (calculated as [ln 2]/0.0664) was 10.4 h; this equates to a 91% loss after 36 h and a 99% loss after 3 d. After 3 h, about 20% of the initial ethanol concentration had been lost.

Exposure to as little as 12.5 $\mu$L EtOH in a 4-L jar significantly reduced lycopene content measured 2 d after treatment by around 25% (Table I). Increasing the amount of ethanol by two- and fourfold resulted in a 44 and 120% increase in the level of inhibition of lycopene content measured after 2 d from 25% to 36% and to 55%, respectively. Increasing the length of exposure from 3 to 6 h had no significant effect on lycopene content measured 2 d after treatment, but had a pronounced effect when measured 4 d after treatment.

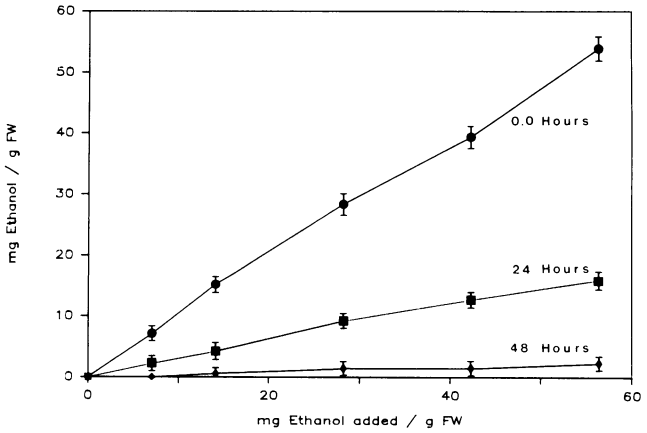


**Figure 2.** Ethanol content of epidermal pericarp discs immediately after alcohol treatment, and after 24 and 48 h in a 10 L $h^{-1}$ flow of humidified air at 20°C. Data represent triplicate samplings. Error bars represent SE of the mean.

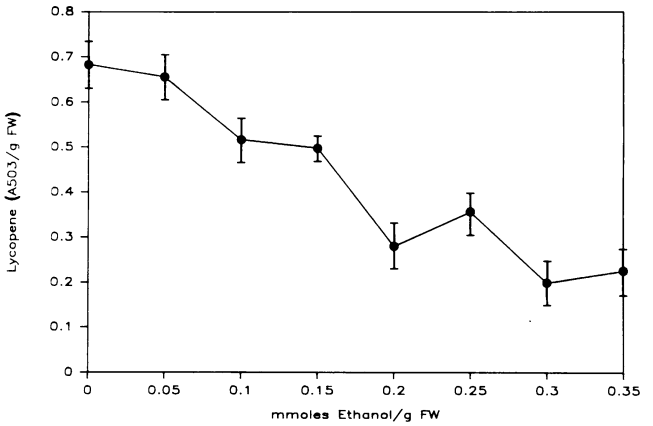


**Figure 3.** Effect of applying 20 $\mu$L of 0.0 to 35% ethanolic solutions directly to the locular surface of pericarp discs on the lycopene content of the discs after ripening at 20°C for 4 d in a flow of humidified air containing 10 $\mu$L/L ethylene. Data represent triplicate samplings. Error bars represent SE of the mean.

**Table I.** *Percent Reduction in Lycopene Content of Tissue Exposed to Ethanol Vapors for 3 or 6 h Compared to Untreated Control Tissue*

Lycopene content was determined after 2 or 4 d in 10 μL $L^{-1}$ ethylene at 20°C; control values were 0.131 and 0.495 $A_{503}$/g FW for d 2 and 4, respectively. Values represent the means and SE of triplicate samples of at least two experiments.

| Ethanol Added to 4-L Jar | Ethanol | Lycopene Content | | | |
|---|---|---|---|---|---|
| | | 3 h exposure | | 6 h exposure | |
| | | d 2 | d 4 | d 2 | d 4 |
| μL | mmol/g | % of control | | | |
| 12.5 | 0.040 | 71 ± 4 | 99 ± 5 | 79 ± 4 | 92 ± 5 |
| 25.0 | 0.078 | 66 ± 5 | 85 ± 4 | 62 ± 3 | 63 ± 6 |
| 50.0 | 0.153 | 46 ± 3 | 56 ± 4 | 45 ± 2 | 44 ± 3 |

**Table II.** *Effect of Ethanol Concentration and Time after Treatment on the Percent of Total Ion Leakage Determined after 0.5 and 6.5 h in Air*

Ion leakage as percent of total, determined after a 2 h soak in 0.3 M mannitol and expressed as percent of total conductivity. Values represent the means and SE of triplicate samples of at least two experiments.

| Ethanol Added to 4-L Jar | Ethanol | Total Ion Leakage | | Ratio of B/A |
|---|---|---|---|---|
| | | Time after removal from ethanol treatment | | |
| | | 0.5 h (A) | 6.5 h (B) | |
| μL | mmol/g | % | | |
| 0.0 | 0.0 | 2.9 ± 0.1 | 5.8 ± 0.2 | 2.0 |
| 400 | 1.2 | 10.1 ± 0.3 | 29.4 ± 0.8 | 2.9 |
| Percent of control | | 348 | 507 | |

The recovery of lycopene synthesis from ethanol inhibition was greater for tissue exposed for shorter periods of time to smaller amounts of ethanol (Table I). Recovery was measured by comparing the lycopene content 2 d after treatment with the content after 4 d. All 3 h exposures showed significant reductions in the level of inhibition, ranging from an almost complete recovery for the 12.5 μL treatment to a 23% recovery for the 50 μL treatment. In contrast, only discs from the 6 h treatment that had been exposed to 12.5 μL showed any significant recovery.

## Ethanol and Ion Leakage

Alcohols are known to perturb cellular membranes; thus, experiments were conducted to investigate the effect of ethanol on ion leakage from treated discs. Ion leakage is a gross measurement of membrane permeability and was measured as the increase in conductivity in a 0.3 M mannitol solution 0.5 h after the completion of the alcohol treatments. Although tissue recovered from alcohol treatment after a few days and proceeded to ripen normally, the rate of ion leakage was greater if the discs were tested 6.5 h after removal from the alcohol treatment than if measured after 0.5 h (Table II).

Exposure to 400 μL EtOH for 3 h increased the rate of ion leakage by about 3.5-fold if measured 0.5 h after treatment, and by 5-fold if measured after 6.5 h in air (Table II). Some of this increase was not caused by the ethanol treatment itself, since ion leakage from control tissue increased 2-fold during 6 h in air. However, ethanol treated discs not only had greater leakage than the controls initially, but the increase was almost 3-fold greater after holding for 6 h in air.

## Interaction between Ethanol and Ethylene

Exposing ethanol-treated discs to μL/L concentrations of ethylene showed that ethylene was able to partially overcome the inhibitory effect of ethanol on lycopene synthesis (Fig. 4). To accentuate differences among treatments, lycopene measurements were made when the control discs that were exposed to the highest concentration of ethylene had just reached maximum ripeness. At this time the control discs not exposed to ethylene had just started to turn red. Exposure to increasing ethanol concentrations from 12.5 to 200 μL for 3 h in a 4-L glass jar resulted in a linear decrease in lycopene content for all ethylene exposures. Discs exposed to ethanol concentrations higher than 200 μL (1.2 mmol EtOH/g FW) failed to respond to ethylene concentrations of 25 to 250 μL/L (data not shown). This agrees with previous reports in which ethylene had no effect on whole fruit, slices, or discs from mature-green tomato fruit exposed to equivalent high concentrations of ethanol (17, 30).

The response of the discs to ethylene was roughly log-linear between 0.25 and 25 μL/L for discs exposed to 25 to 100 μL ethanol, and log-linear between 0.0 and 25 μL/L for discs exposed to 200 μL/L ethanol (Fig. 5). In the absence of added ethylene, discs exposed to 25 to 100 μL/L ethanol started to ripen days before discs exposed to 200 μL or higher concentrations of ethanol. Ethylene is produced autocatalytically by ripening tomato tissue, and the internal ethylene concentration probably accumulated to stimulatory levels in discs exposed to 100 μL or less ethanol. Discs exposed to 200 μL ethanol ripened much slower and probably did not accumulate stimulatory levels of ethylene until much later. On the basis of their ripening behavior, it appeared that discs exposed

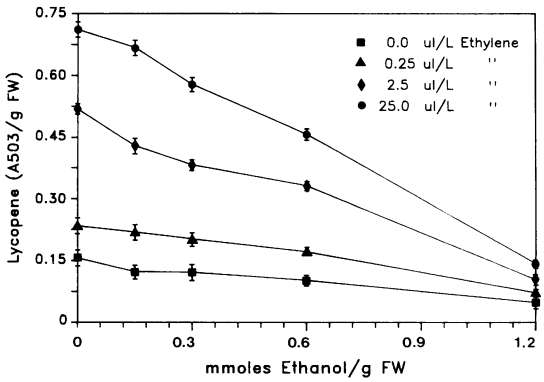


**Figure 4.** Effect of ethanol on the lycopene content of discs ripened at 20°C for 4 d in atmospheres containing from 0.0 to 25 μL/L ethylene. Discs were exposed to 0.0 to 200 μL ethanol for 3 h in a 4-L jar before ripening. Data represent triplicate samplings. Error bars represent SE of the mean.

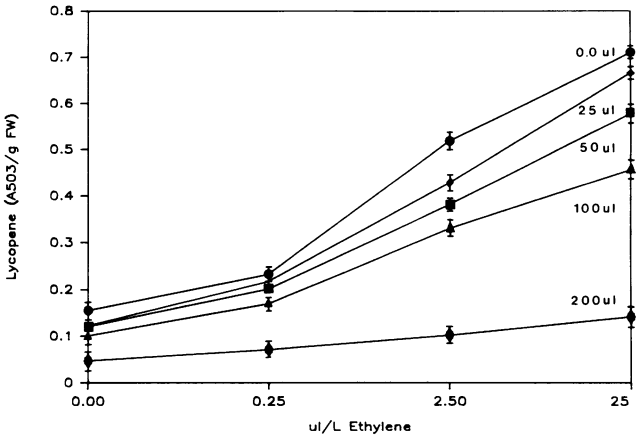


**Figure 5.** Effect of ethylene on the lycopene content of discs exposed to 0.0 to 200 μL ethanol for 3 h in a 4-L glass jar and ripened at 20°C for 4 d. Data from Figure 2. Data represent triplicate samplings. Error bars represent SE of the mean.

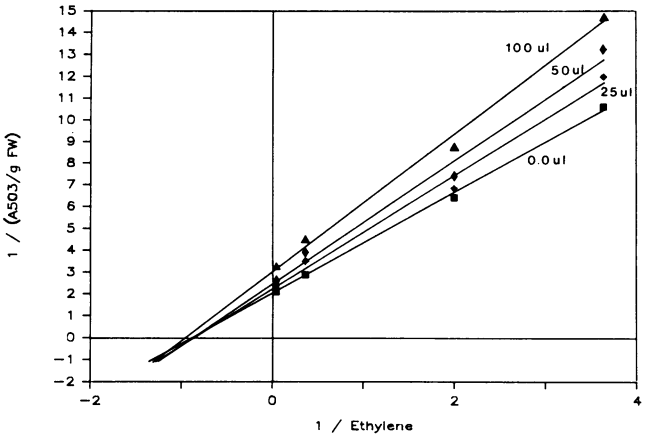


**Figure 6.** Lineweaver-Burk plot of ethylene *versus* lycopene content of discs exposed to 0.0 to 100 μL ethanol and ripened in atmospheres containing from 0.0 to 25 μL/L ethylene.

to low concentrations of ethanol contained an effective concentration of about 0.25 μL/L ethylene.

Regression lines that had been fitted to lycopene content *versus* ethylene exposure data that had been transformed for a Lineweaver-Burk plot (*i.e.* 0.25 μL/L ethylene was added to each ethylene concentration) had regression coefficients ($r$) of better than 0.95 (Fig. 6). The lines for the 25 to 100 μL ethanol treatments intercepted the $x$ axis in accordance with an analysis of noncompetitive interaction between ethanol and ethylene on ethanol inhibition of lycopene synthesis. The line for the 200 μL ethanol treatments was far removed from the lines of the lower ethanol concentrations and was not shown in the figure. Known competitive inhibitors of ethylene action (*e.g.* carbon dioxide) produced the classic Lineweaver-Burk plot with intersection of the lines on the $y$ axis (3).

### Effects of Other Aliphatic Alcohols

Other alcohols also significantly inhibited lycopene synthesis at concentrations which did not significantly increase ion leakage (Fig. 7). Of the three alcohols tested, ethanol showed the greatest inhibition of lycopene content at concentrations that did not stimulate ion leakage. Ethanol inhibited lycopene synthesis by 85% at a concentration that did not significantly increase ion leakage. On a molar basis, the toxicity of the alcohols as measured by the highest concentration that did not significantly increase ion leakage was similar for methanol and ethanol, but much higher for $n$-propanol.

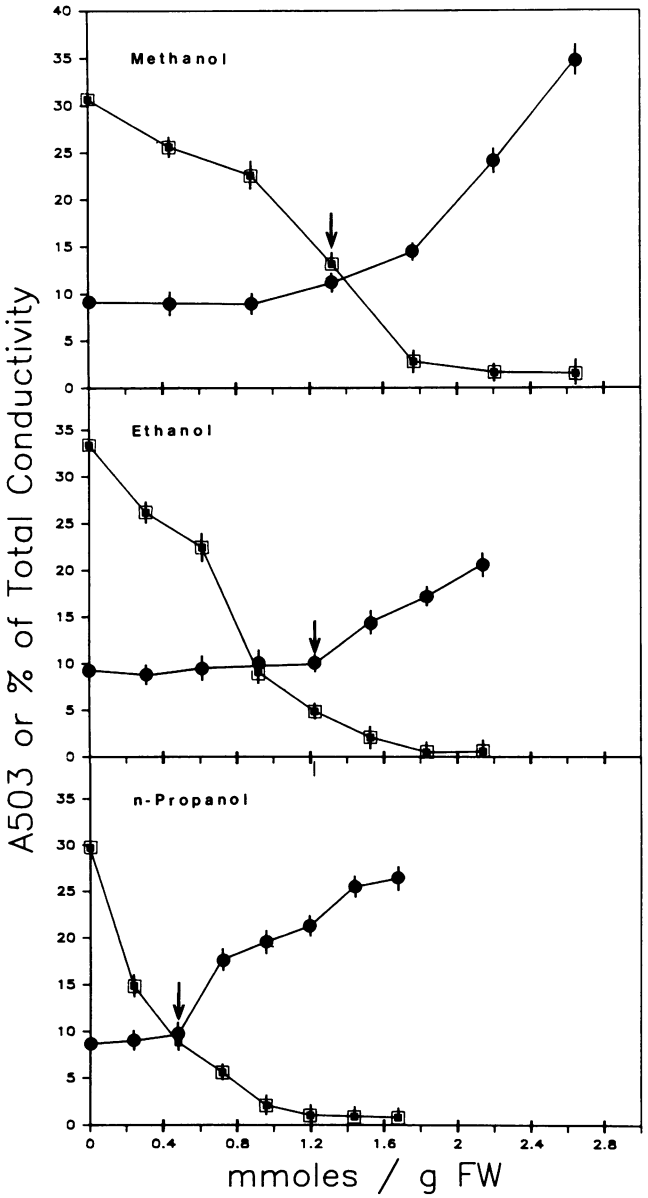


**Figure 7.** Percent of total conductivity (●) and lycopene content (■) as $A_{503}$ $g^{-1}$ FW of discs exposed to various concentrations of methanol, ethanol, and $n$-propanol for 3 h in a 4-L glass jar. The arrow indicates the highest alcohol concentration at which conductivity was not significantly different from the untreated control. Discs were ripened at 20°C for 5 d in 20-L glass jars that were flushed with humidified air containing 10 μL/L ethylene. Data represent duplicate samplings from at least three experiments. Error bars represent SE of the mean.

Regression analyses were conducted on a number of attributes of the alcohols and their effects on ion leakage and lycopene synthesis (Table III). The highest significant correlation was between the tissue concentration (mmol/g FW) required for 50% reduction in lycopene content and the mol wt of the alcohols. Although highly correlated ($r = 0.99+$), this relationship is tenuous because only three alcohols were tested.

## DISCUSSION

Although ethanol can be toxic to plant tissue, deleterious concentrations are rarely if ever encountered naturally because toxic concentrations are usually many-fold higher than those that accumulate as the result of endogenous synthesis. In a recent survey of over 50 plant species, Kimmerer and MacDonald (19) found that after 4 and 24 h under $N_2$, leaves from herbaceous plants contained an average of 4.1 and 8.3 $\mu$mol ethanol $g^{-1}$ FW, respectively, while leaves from woody plants contained an average of 15 and 74 $\mu$mol $g^{-1}$, respectively. Another study found ethanol concentrations of 28 to 93 $\mu$mol $g^{-1}$ in legume seeds germinated for 24 h under water (7). In a study using anaerobic gas mixtures to simulate flooding, sweet potato roots were found to accumulate 20 or 36 $\mu$mol ethanol $g^{-1}$ after 48 h in $N_2$ or $CO_2$, respectively (5). Ethanol concentrations of 0.15 to 0.5 $\mu$mol $g^{-1}$ were found in the vascular cambium of trees growing in air (20). Aerobically ripening apple fruit contained 0.4 to 1.3 $\mu$mol ethanol $g^{-1}$, which increased to over 22 $\mu$mol $g^{-1}$ after 4 d in $N_2$ (25). In one study, ripening tomato fruit were found to contain 2.7 to 4.3 $\mu$mol ethanol $g^{-1}$ (11), while it was undetected in a recent study in this laboratory (17). Mature-green tomato fruit accumulated slightly more than 30 $\mu$mol ethanol $g^{-1}$ after 48 h in $N_2$ (17); a concentration which effectively inhibited ripening (17, 30).

An extensive literature survey revealed that deleterious effects of ethanol were only observed at concentrations 12- to 200-fold higher than those found in flooded plants (14). The applied ethanol concentration that produced 50% mortality in cold-hardened cereals ranged from 187 to 520 $\mu$mol $g^{-1}$ FW, while the concentrations accumulated during 7 d of anaerobiosis were less than 60 $\mu$mol $g^{-1}$ (1). Ethanol concentrations 45-fold greater than endogenous levels produced during flooding slightly inhibited shoot elongation (*i.e.* 515 *versus* 11 $\mu$mol $g^{-1}$), but were not toxic to germinating grass seeds (27). Apple fruit were not damaged by long term exposure to ethanol concentrations below 26 $\mu$mol $g^{-1}$ (9).

While plant tissues are capable of ethanol metabolism (8), it has not always been detected (23) and ethanol loss probably occurs predominantly through evaporation. Ethanol metabolism by tomato tissue has been documented (24), but in the present study metabolism was probably insignificant when compared to losses through diffusion. The ethanol decay curve resembled a simple diffusion process; it was similar in form to that of ethanol loss from blueberries at 0°C (28), and it was similar for ethanol concentrations induced in the tissue by anaerobiosis and for the many-fold higher concentrations induced by exposure to ethanol vapor (17).

### Interaction between Ethanol and Ethylene

Ethanol concentrations between 0.0 and 0.6 mmol $g^{-1}$ appeared to inhibit tomato fruit ripening by interfering with ethylene action in a noncompetitive fashion (Fig. 6). Ethylene was much less effective at overcoming ethanol inhibition at ethanol concentrations above 0.6 mmol $g^{-1}$, and the Lineweaver-Burk plot of those data did not intersect the plot of data from lower ethanol concentrations (see 200 $\mu$L line in Fig. 5). This suggests that two possible mechanisms may be involved in ethanol inhibition of tomato fruit ripening. At concentrations below 0.6 mmol $g^{-1}$, ethanol appears to be directly affecting ethylene action, whereas at concentrations above 1.2 mmol $g^{-1}$, ethanol appears to have a nonspecific toxic effect. This is shown by the fact that while lycopene synthesis steadily declined to near zero as the ethanol concentration approached 2 mmol $g^{-1}$, ion leakage only increased at concentrations above 1.2 mmol $g^{-1}$ (Fig. 7). Most of the inhibition of lycopene synthesis occurred at concentrations far below those causing increased ion leakage. Inhibition of ethylene action by the lower ethanol concentrations is probably of physiological importance since it occurs at concentrations which are naturally found in tissue.

### Effect of Other Aliphatic Alcohols

Ethanol inhibition of tomato fruit ripening was not unique. Both methanol and *n*-propanol also inhibit ripening (Fig. 7) and the degree of inhibition was related to the mol wt of the alcohols (Table III). This agrees with other reports showing that the biological effectiveness of a series of *n*-alcohols was related to the chain length or mol wt of the alcohols (4, 21, 22, 31, 32). For example, the senescence retarding effect of alcohols in oat leaves increased in a log-linear fashion from ethanol to *n*-octanol, with a log increase in effectiveness

**Table III.** *Alcohol Concentration that Resulted in a 50% Inhibition of Lycopene Content, and the Alcohol Concentration and Lycopene Content at the Highest Alcohol Concentration that Did Not Cause a Significant Increase in Conductivity Over the Control*

Values represent the means and SE of triplicate samples of at least two experiments.

| Alcohol | Concentration at 50% Inhibition | Concentration Before Increase in Conductivity | Lycopene Content Before Increase in Conductivity | |
|---|---|---|---|---|
| | *mmol/g FW* | | $A_{503}$/*g FW* | *% of control* |
| Methanol | 1.25 ± 0.06 | 1.32 ± 0.04 | 0.26 ± 0.02 | 43 |
| Ethanol | 0.76 ± 0.04 | 1.23 ± 0.05 | 0.10 ± 0.01 | 15 |
| *n*-Propanol | 0.23 ± 0.02 | 0.46 ± 0.03 | 0.17 ± 0.01 | 28 |

related to a linear increase in mol wt of the alcohol (31). All three alcohols used in the present study appeared to be affecting a common step in ethylene action.

Significant inhibition of ripening by all three alcohols occurred at concentrations which were many times lower than those which increased ion leakage, and the inhibition curves were roughly the same shape (Fig. 7). Of the three alcohols tested, ethanol showed the greatest inhibition, with a significant 25% reduction in lycopene content at about 3% of the ethanol concentration that caused an increase in ion leakage (Table I). When the highest concentration of the alcohols that inhibited ripening without increasing ion leakage were compared, ethanol again proved the most effective at inhibiting ripening (57% reduction for 1.3 mmol methanol $g^{-1}$, 85% for 1.2 mmol ethanol $g^{-1}$, and 72% for 0.45 mmol *n*-propanol $g^{-1}$). The pronounced activity at these concentrations implies that the alcohols were not simply having a gross effect on changing the permeability of cellular membranes, but were probably selectively affecting some common step in ethylene action.

### Effect of Duration of Exposure

The observation that the rate of ion leakage continued to increase even as the tissue concentration of ethanol decreased, shows that ethanol has a residual effect that persists even after its removal. Rather than just physically perturbing the membranes by its presence and grossly altering membrane permeability, it appears that the alcohols actually affected some physiological process related to ethylene action that has a longer half-life for recovery than the half-life for ethanol loss.

Even though tissue ethanol content did not increase between the 3 and 6 h exposure periods (97% was absorbed within 3 h) the additional 3 h exposure had a significant long-term effect on lycopene synthesis (Table I). Since the half-life for ethanol loss was around 10.4 h, only around 20% of the internal ethanol would have been lost from discs during the 3 h that separated the two lengths of exposure. The continued exposure did not have much of an effect after 2 d, but had a pronounced effect on cumulative lycopene synthesis 4 d later (Table I). The concentration × time dependency of ethanol inhibition is further confirmed by results from studies with whole fruit (17, 30). Exposure to ethanol vapors that induced around 70 $\mu$mol $g^{-1}$ for 12 h (30) or 24 h (17) effectively inhibited ripening. In contrast, induction of 5- to 10-fold higher tissue concentrations were required to induce a similar level of inhibition with a 3 h exposure.

At higher concentrations, the ethanol effect on ion leakage did not immediately dissipate upon removal from the treatment, but continued to increase for a period of at least 6 h (Table II). The observation that discs recovered from ethanol inhibition and ripened normally showed that the long-term effects of ethanol are reversible. Since ion leakage greatly increases upon ripening, small differences in the stage of ripeness confounded measurements of leakage in recovered, ripening discs. The accompanying high degree of variability in these measurements prevented meaningful analysis of the short-term effect of ethanol on leakage from ripening discs (data not shown).

### Conclusion

A possible explanation for the observed inhibitory effects of alcohols are the documented effects of alcohols on cellular membranes. Aliphatic alcohols have been shown to inhibit photophosphorylation in chromatophores and chloroplasts, and respiration and phosphorylation in mitochondria (32). It was suggested that the alcohols penetrated into the lipid bilayers, produced changes in the structural arrangement of the lipids and, consequently, increased permeability (21). This possibility is supported by a recent study that showed that membrane fluidity and permeability increased exponentially in yeast plasma membrane with increasing ethanol content (16). In another study it was shown that ethanol disrupted the phospholipid bilayer immediately adjacent to the $(Na^+ + K^+)$-ATPase and caused its irreversible denaturation without significantly disrupting the protein structure of the membrane (10). The functional organization of the inner mitochondrial membrane was also disturbed by alcohols which disrupted lipid-protein bonds (22). Ethanol appears able to cause structural changes in cell membranes that result in increased permeability and denaturation of associated proteins.

Previous reports of the effect of alcohols on various biological processes have correlated the effectiveness of the alcohol with its lipophilia (22, 31). Satler and Thimann (31) showed that the effectiveness of a series of aliphatic alcohols in delaying leaf senescence was paralleled by their ability to open stomata. They proposed the mode of action was probably through increased membrane permeability to potassium.

Aliphatic alcohols are known to accumulate preferentially in lipids, especially at the interface between the polar groups in cell membranes (16). The extent of partitioning is governed by the composition of the membrane. This could partially account for the differential effects of ethanol concentrations that reduced ethylene action and the much higher concentrations that increased ion leakage. The membranes associated with ethylene action could be comprised of lipids that would favor ethanol accumulation so that more disruption of the membrane would occur at lower concentrations. This idea is supported by the fact that there are known differences in ethanol sensitivity of various mammalian membranes caused by variations in their lipid composition (6).

I propose that alcohols affect ethylene action by permeating into the lipid bilayer of a selected membrane system associated with ethylene action, distorting the structural arrangement and causing increased denaturation of the ethylene receptor cite. The perturbation of the membrane appears to have been slight since there was a pronounced reduction in ethylene action and lycopene synthesis at concentrations much lower than those that caused increased permeability. Also, the rate of denaturation was probably slow since prolonged ethanol exposure resulted in increased inhibition.

Ethanol concentrations that inhibited tomato fruit ripening were generally higher than those found naturally in many tissues. However, the time by concentration dependency of ethanol on ethylene action could indicate that ethanol has a significant effect at the low concentrations encountered in stressed tissue. Since ethanol is effective at concentrations which are known to be produced during stress, fruit ripening

and low oxygen storage, ethanol may have an important physiological role in the response of plants to stress and fruit ripening, and could explain some of the beneficial effects of controlled atmosphere storage. Effective ethanol concentrations can be induced by short periods of anaerobiosis or by exposure to ethanol vapors and could be used to augment current postharvest treatments to control fruit ripening.

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