Inhibition or Promotion of Tomato Fruit Ripening by Acetaldehyde and Ethanol is Concentration Dependent and Varies with Initial Fruit Maturity

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ABSTRACT. 'Castlemart' tomato (*Lycopersicon esculentum* Mill.) pericarp discs were used to study the physiological effects of acetaldehyde and ethanol on fruit ripening. Short-term exposure of discs from mature-green fruit to acetaldehyde vapors on a fresh mass basis ($\leq 500 \mu\text{g}\cdot\text{g}^{-1}$) or ethanol vapors ($\leq 3 \text{ mg}\cdot\text{g}^{-1}$) promoted ripening, while higher concentrations inhibited ripening. Discs from mature-green fruit absorbed greater amounts of ethanol and produced significantly higher concentrations of acetaldehyde than discs from breaker fruit. Ripening was promoted by ethanol when the discs were unable to retain or produce a certain level of acetaldehyde. Inhibition of ripening by 4 hours of exposure to ethanol ($6 \text{ mg}\cdot\text{g}^{-1}$) was almost completely abolished by hypobaric treatments (18 kPa for 24 hours). However, acetaldehyde-induced ripening inhibition (2 days exposure to $180 \mu\text{g}\cdot\text{g}^{-1}$) was only slightly reduced by vacuum. Concentrations of acetaldehyde and ethanol that inhibited ripening reduced C_2H_4 production, whereas lower concentrations of acetaldehyde and ethanol that promoted ripening increased C_2H_4 production. Application of 4-methylpyrazole, an alcohol dehydrogenase inhibitor, enhanced acetaldehyde-induced ripening inhibition and reduced ethanol-induced ripening inhibition or promotion at all concentrations of acetaldehyde and ethanol tested. The inhibition or promotion of ripening of excised tomato pericarp discs by ethanol and acetaldehyde depended on initial fruit maturity, applied volatile concentration, and duration of exposure.

Ethanol (EtOH) is produced in many plants under anaerobic (Kimmerer and Kozlowski, 1982) or aerobic (Chang et al., 1983) atmospheres and can be isolated from all color stages of mature tomato fruit (Gustafson, 1934). Acetaldehyde (AA) is an aroma constituent in most plant tissues, and endogenous AA levels increase in climacteric fruit as they ripen (Fidler, 1968; Gustafson, 1934; Janes and Frenkel, 1978). The enzyme alcohol dehydrogenase (ADH) rapidly metabolizes AA to EtOH in plants (Oppenheim and Castelfranco, 1967).

The quality of many commodities can be altered by exposure to AA and EtOH. Exposure to AA vapors improved the organoleptic quality of table grapes (*Vitis vinifera*, L.) (Pesis and Frenkel, 1989), strawberries (*Fragaria vesca* L.) (Morris et al., 1979), blueberries (*Vaccinium corymbosum* L.), and tomatoes (*Lycopersicon esculentum*) (Paz et al., 1981). In blueberry and tomato fruit, applied AA vapors increased reducing sugars 2.5- and 1.4-fold, respectively (Paz et al., 1981). Removal of astringency from persimmon (*Diospyros kaki* Thunb.) fruit held under modified atmospheres was correlated with AA accumulation (Pesis and Ben-Arie, 1984). Applied AA vapors promoted the ripening of blueberry, tomato, and pear (*Pyrus communis* L.) fruit (Paz et al., 1981; Janes and Frenkel, 1978). The ripening of pear fruit was stimulated by AA vapors, even when C_2H_4 was removed hypobarically or C_2H_4 action was antagonized chemically (Janes and Frenkel, 1978). While AA and C_2H_4 actions appeared to be independent at low concentrations, exposure to higher AA concentrations slightly inhibited C_2H_4 production and ripening.

The respiration of apple (*Malus domestica* Borkh.), blueberry, potato (*Solanum tuberosum* L.), orange (*Citrus sinensis* L. Osbeck), and strawberry fruit was stimulated by AA vapors (Fidler, 1968; Janes et al., 1978). Application of AA inhibited softening and decreased C_2H_4 production in peach (*Prunus persica* L. Batsch) (Lurie and Pesis, 1992), while it dramatically increased C_2H_4 production from kiwifruit (*Actinidia deliciosa* L.) (Mencarelli et al., 1991). Alteration in ripening-related parameters are commonly observed with climacteric fruit; however, generally only acidity and soluble solids appear to be affected by AA treatments in nonclimacteric fruit (Morris et al., 1979; Pesis and Avissar, 1989a, 1989b; Pesis and Frenkel, 1989). Nonetheless, application of AA to intact grape berries increased CO_2 production and reduced C_2H_4 production (Pesis and Marinansky, 1992). Addition of 1-aminocyclopropane-1-carboxylic acid (ACC) to the cut surface of grape berry halves increased C_2H_4 production 10-fold, and this increase could be inhibited 80% by AA. Ethanol applied at the same concentration as AA did not reduce C_2H_4 production or inhibit the conversion of ACC to C_2H_4 , and Pesis and Marinansky (1992) concluded that AA, not EtOH, inhibited conversion of ACC to C_2H_4 in grape berries. Supporting this conclusion, Hewage et al. (1995) reported that AA, but not EtOH, inhibited the ripening of banana (*Musa* AAA, Cavendish subgroup) fruit. Burdon et al. (1996) also reported that AA was effective in inhibiting ethylene biosynthesis in mango (*Mangifera indica* L.) fruit.

Exogenously applied or endogenously synthesized EtOH inhibited tomato fruit ripening at various maturity stages (Kelly and Saltveit, 1988; Saltveit and Mencarelli, 1988) without affecting subsequent quality (Saltveit and Sharaf, 1992). Treatment with EtOH also reduced the capacity for aminoethoxyvinylglycine (AVG)-treated discs to convert exogenously applied ACC to C_2H_4 , and ACC accumulated while ACC oxidase activity was reduced. Ripening inhibition appeared to act through an inhibition of C_2H_4 synthesis and action (Saltveit and Mencarelli, 1988). Saltveit (1989) then applied classical Lineweaver-Burk kinetics in similar experiments and concluded that tomato discs exposed to EtOH for 3 h at 34 to 79 $\text{mg}\cdot\text{g}^{-1}$ displayed noncompetitive inhibition of C_2H_4

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action. Therefore, exogenously applied or endogenously synthesized EtOH, which inhibited tomato fruit ripening, also suppressed C_2H_4 action noncompetitively (Saltveit, 1989). More recently, short-term static induction of AA via EtOH vapors applied in concert with 4-methylpyrazole (4-MP), an inhibitor of ADH (Blomstrand et al., 1979; Lelback, 1969), or continuous AA vapor applications were found to inhibit the ripening of excised tomato discs (unpublished results) and slightly inhibit ripening in whole tomato fruit (Pesis and Marinansky, 1993).

Tomato discs provide an ideal experimental system because discs from one fruit represent genetically uniform and environmentally homogeneous samples. Twenty or more discs can be excised from the equatorial region of one fruit. Furthermore, ripening of discs is similar to ripening of whole fruit, with development of a respiratory and C_2H_4 climacteric, starch degradation and production of sugars, chlorophyll degradation, lycopene and carotene synthesis, softening, and production of characteristic flavor and aroma (Campbell et al., 1990; Saltveit and Mencarelli, 1988). Additionally, since many ripening-related phenomena are sensitive to rates of gas diffusion into and out of tissue, another advantage of discs is that their cut surfaces minimize the resistance to gas diffusion.

Research reported in this paper used excised tomato pericarp discs as a model system to study the short- and long-term physiological effects of applied EtOH and AA vapors on the promotion and inhibition of fruit ripening.

Materials and Methods

PLANT MATERIALS. 'Castelmar' tomato fruit were hand harvested from plants grown under normal cultural practices at the Univ. of California Vegetable Crops Field Facility. Where noted, 'Dombito' and 'Jackpot' tomato fruit were used in a few experiments. Unblemished, uniformly shaped fruit (≈ 160 g) were used to prepare discs.

PREPARATION OF EPIDERMAL PERICARP DISCS. Fruit were washed with a dilute bleach solution (1:20 v/v), rinsed three times with sterile deionized water, and air dried under a sterile laminar flow hood. Epidermal pericarp discs were cut from the equatorial regions with a 1.4-cm-diameter cork borer, and adhering locular tissue was trimmed away to produce 2-, 3-, or 4-mm-thick discs. Discs were rinsed three times with sterile deionized water, carefully blotted dry, blocked by intensity of green color among treatments (Saltveit, 1989), and placed epidermis side down into sterile petri dishes or deep-well culture plates. Before and after disc extraction, fruit were carefully scrutinized for exact mature-green (MG) status. The maturity of each tomato fruit was classified as follows: MG2 tomatoes were 6 to 10 d from breaker stage with little or no locular jelly, but seeds were not cut with a knife due to the mature and softened locular tissue; MG3 tomatoes were 2 to 5 d from breaker stage with well-developed locular jelly and no visible red color within the columella; and MG4 tomatoes were 1 or 2 d from the breaker stage with internal red color development around the columella or within the jelly-like locule. Discs were always placed at 20 °C overnight (≈ 18 h) in a 20 L·h⁻¹ flow of humidified air to allow for wound C_2H_4 dissipation (Abeles et al., 1992).

COLOR MEASUREMENTS. Subjective evaluations of ripeness and objective measures of lycopene content were correlated with light reflectance measurements to establish the relationship among these three measures of ripeness. Discs, 2, 3, or 4 mm thick (1.4 cm in diameter, $n = 36$), were prepared from a number of fruit ranging in maturity from MG2 to red-ripe. The discs were individually weighed (0.385 ± 0.039 , 0.506 ± 0.036 , and 0.622 ± 0.038 g for 2-, 3-, and 4-mm-thick discs, respectively), and their L^* , a^* , and b^* CIE values were recorded with a Minolta chromameter. Each disc

was then homogenized in 10 mL acetone, centrifuged at 3000× *g* for 5 min, and the absorbance of the supernatant measured against an acetone blank at 503 nm on a spectrophotometer. Lycopene concentrations, on a fresh mass basis, were positively correlated with a^*/b^* values, and the best curvilinear equation [$\mu\text{g}\cdot\text{g}^{-1} = 10.35(a^*/b^*)^2 + 5.54(a^*/b^*) + 1.29$; $r^2 = 0.96$] was obtained with 4-mm-thick discs (Beaulieu, 1996; Beaulieu et al., 1997). Calibrating the chromameter to either the red or white standard color tile yielded identical curves, but the white calibration produced on average a^*/b^* values 0.2 units higher (data not shown).

All data presented are from representative experiments, which were repeated at least once. Fisher's least significant differences (LSDs) were calculated at $P = 0.01$ or 0.05 using pooled standard error of the differences between means according to Snedecor and Cochran (1980).

VOLATILE TREATMENTS. After dissipation of wound-induced C_2H_4 , petri dishes or culture plates of discs were transferred to a sterile hood, uncovered, and placed into previously sterilized 4-L glass jars. A folded filter paper nested in a 15×60-mm plastic petri dish was placed beside the culture plate and an amount of water, EtOH or AA, calculated on a fresh mass basis, was pipetted onto the filter paper. The jar was then immediately capped with a Teflon-covered rubber stopper and sealed for 4 h. The culture plates were then removed from the jars, covered, and transferred to 20-L glass jars at 20 °C with a 20 L·h⁻¹ flow of humidified air. Alternatively, after dissipation of wound-induced C_2H_4 , discs were placed inside 1-L glass jars and sealed with Teflon septa lids. Water, EtOH, or AA was injected through the septa lid onto a 4.25-cm Whatman filter paper, which was taped inside the rim of the jar, and the jar was sealed for 4 h. Tissue was then removed and transferred to 20-L glass jars at 20 °C ventilated with a 20 L·h⁻¹ flow of humidified air. All EtOH treatments were visually determined to have volatilized before the end of the 4 h treatment period, and all filter papers that had been dampened with EtOH were dry to the touch when treatment jars were opened.

Acetaldehyde vapors were applied in a flow of humidified air similar to the method of Janes et al. (1978). AA vapors slowly diffused through a capillary tube protruding from the sealed end of a 20-mL vial, partially filled with liquid AA, into the 500-mL mixing flask at 20 °C. The flow of air through the mixing flask was adjusted to achieve specific levels of AA. Fruit discs were exposed to flows of humidified air enriched with AA for various durations in 20-L glass jars. Treatment levels were verified before and during an experiment by injecting 1-mL headspace samples into a gas chromatograph (GC), which was calibrated against a standard curve derived from known serially diluted AA standards. After treatment, culture plates or petri dishes containing discs were transferred to 20-L glass jars at 20 °C ventilated with a 20 L·h⁻¹ flow of humidified air. In one series of experiments, discs were exposed to an 18-kPa vacuum in a plastic desiccator. The atmosphere in the chamber was humidified with moist paper towels and scrubbed for CO₂ with KOH pellets. A water aspirator connected to a pressure regulator maintained the vacuum for 24 h.

VOLATILE GC DETERMINATIONS. The GC headspace method of Davis and Chance (1969) was used to rapidly sample volatiles from tomato discs. Briefly, discs were removed from treatments and immediately placed into 10-mL glass syringes drawn to 7 mL, the tip was sealed with a rubber serum stopper, the syringes were frozen at -20 °C and heated in a water bath at 75 °C for 1 h, and then a 1-mL gas sample was removed and analyzed by GC. The GC was equipped with a 183-cm-long 5% carbowax 20M 60/80 carboxpack blood alcohol column held at 85 °C with the injection port at 150 °C and the detector at 250 °C. The output from the GC was used to

calculate the tissue concentration using a standard curve produced by injecting 1-mL gas samples from a calibrated series of known serially diluted standards. The amount of standard solution put in each syringe was equal to the average disc mass. Complete volatilization of AA and EtOH would not occur under these assay conditions, and the amount analyzed would be proportional to the concentration in the sample and not an absolute measure of the total amount in the sample. Using a mass of standard solutions equal to the mass of the discs in the construction of the standard curve allowed us to construct a standard curve in which the measured proportional amount of AA and EtOH could be related back to an absolute amount in the discs. All values are reported on a fresh mass basis.

ETHYLENE DETERMINATIONS. Ethylene was determined from within static vessels sealed with Teflon lids or deep-well culture plates (sealed with septa sheets that were covered and clamped tight with Plexiglas plates with 0.5-mm drilled holes for needle syringe insertion). Headspace gas samples (1 mL) were injected into a GC equipped with a sodium chloride modified Alumina F1 60-80 column (Carle Instruments Inc.) and an flame ionization detector as previously described (Saltveit and Yang, 1987).

RIPENING MEASUREMENTS. Chromameter readings from fruit discs were superior to the subjective evaluations (i.e., color charts) of ripening because the nondestructive chromameter readings could be taken from specific regions of the fruit and the location on the fruit from which the discs are excised can determine the timing of ripening (Brecht, 1995). We also observed that disc thickness influenced the correlation between lycopene concentrations and chromameter a^*/b^* readings. We therefore used 4-mm-thick discs in all subsequent experiments because that thickness was found to have the highest correlation ($r^2 = 0.96$) between chromameter a^*/b^* values and lycopene content (Beaulieu et al., 1997).

Results and Discussion

ETHANOL APPLICATIONS. During our studies of the inhibition of tomato fruit ripening by EtOH, we observed that low EtOH concentrations actually promoted fruit ripening (Beaulieu et al., 1997; Saltveit, 1989). When ripening was monitored daily in pericarp discs excised from MG2, MG3, and MG4 fruit, the effect of a 4-h exposure to EtOH vapor at 0.8 to 4.7 mg·g⁻¹ was found to vary with EtOH concentration and fruit maturity (Fig. 1). Not only did none of the EtOH concentrations inhibit the ripening of MG2 disks, but EtOH concentrations below 4.7 mg·g⁻¹ actually significantly increased ripening. As fruit matured and approached the respiratory and ethylene climacteric (i.e., MG4), higher EtOH concentrations became inhibitory to ripening so that in MG4 fruit only 0.8 and 1.6 mg·g⁻¹ promoted ripening while 2.4 to 4.7 mg·g⁻¹ caused a progressively greater inhibition of ripening. In discs from MG3 fruit, ripening was increasingly promoted by EtOH concentrations below 3.2 mg·g⁻¹, while concentrations above 4.7 mg·g⁻¹ inhibited ripening.

When data in Fig. 1 for selected dates were arranged so that the controls for each maturity stage had similar levels of lycopene, the dissimilar promotive and inhibitory effects of EtOH was easily seen (Fig. 2). The ripening of all maturities was promoted ≈2.5-fold by exposure to EtOH at 0.8 mg·g⁻¹ for 4 h. Increasing the EtOH concentration beyond 0.8 mg·g⁻¹ did not markedly increase the rate of ripening of any of the maturities, as shown by the similar maximum levels of lycopene for all maturities and EtOH treatments. However, the ripening promotive effect of EtOH showed a significant decline as the EtOH concentration increased to 1.6, 2.4, and 4.7 mg·g⁻¹ for MG4, MG3, and MG2 discs, respectively.

Tissue of different maturity absorbed EtOH vapors and produced AA differentially. After an 8-h exposure to EtOH at 6.3

mg·g⁻¹, preclimacteric MG3 discs had absorbed 15% more EtOH than climacteric MG4 discs (5.4 ± 0.5 versus 4.7 ± 0.6 mg·g⁻¹) and had produced 38% more AA (112 ± 18 versus 81 ± 14 ng·g⁻¹). Since the tissue AA, and not the EtOH, concentration is responsible for inhibiting ripening (Beaulieu et al., 1997), the increased level of EtOH needed to inhibit the ripening of MG4 tissue could result from its reduced capacity to absorb EtOH and produce AA. Also, the level of EtOH resulting from continuous AA exposure in MG4 discs (Beaulieu et al., 1997) actually fell within the range observed to promote ripening during a 4-h application of EtOH at 3.2 mg·g⁻¹ (Fig. 1). Discs exposed to EtOH at 0.0 to 3.2 mg·g⁻¹ for 4 h had tissue levels of AA immediately after treatment that increased quadratically from 0.7 to 75 µg·g⁻¹ as EtOH increased from 0.0 to 1.6 mg·g⁻¹ and then remained relatively constant at 80 ± 8.7 µg·g⁻¹ as EtOH increased from 1.6 to 3.2 mg·g⁻¹.

It was difficult to compare the responses of our model disc system with previously reported results because whole tomatoes or transverse slices were used instead of pericarp discs. For example, Saltveit (1989) reported ripening inhibition in tomato pericarp discs using objective measures of lycopene content and relatively high (43 mg·g⁻¹) levels of EtOH for 3 h. In contrast, Saltveit and Sharaf (1992) used whole fruit and subjective color measurements. Saltveit and Mencarelli (1988) and Kelly and Saltveit (1988) found inhibition of ripening with longer periods of exposure to EtOH (12

Fig. 1. Effect of maturity and concentration of applied ethanol vapor on subsequent ripening of excised tomato pericarp discs. Fruits were scrutinized for their exact ripeness stage (i.e., MG2, MG3, or MG4) at the time of disc excision. Discs were exposed for 4 h to ethanol vapor on a fresh mass basis at 0 to 4.7 mg·g⁻¹ in a 4-L glass jar. Chromameter a^*/b^* readings ($n = 4$) were used to calculate lycopene concentrations on a fresh mass basis and 5% LSD values.

Fig. 2. Effect of maturity and concentration of ethanol vapor applied on a fresh mass basis on ripening of excised tomato pericarp discs. Data from Fig. 1 are arranged so that the controls (EtOH at 0 mg·g⁻¹) have similar levels of lycopene content on a fresh mass basis; i.e., day 7 for MG2, day 6 for MG3, and day 4 for MG4.

or 24 h). Saltveit and Mencarelli (1988) found that EtOH at 1.6 and 3.2 mg·g⁻¹ inhibited ripening, as measured by subjective color evaluations, after a comparable exposure period (3 or 6 h) using equatorial tomato slices rather than discs. Since there are differences in absorption capability between tomato pericarp discs and equatorial slices containing locule materials, the slices may have absorbed a greater concentration of EtOH than the discs in the same amount of time. In addition, since the exact maturity of the mature-green fruit was not reported, results of these studies cannot be compared to the maturity-dependent effects we report in this manuscript.

ACETALDEHYDE APPLICATIONS. The maturity of the discs also influenced their response to AA. Exposure to AA at 100 to 500 μg·g⁻¹ for 4 h promoted ripening of discs from MG3 and MG4 fruit (Fig. 3); but, unlike the effect of maturity on EtOH action, the ripening of discs from MG4 fruit was promoted to a greater extent than discs from MG3 fruit. Also, none of the AA concentrations inhibited the ripening of MG3 or MG4 discs. All AA concentrations that significantly promoted ripening also increased C₂H₄ production, which peaked 1 d before the controls (data not shown). Exposure to AA vapors at 250 μg·g⁻¹ for 4 h had no significant effect on the ripening of MG2 discs (data not shown).

Since the short-term effect of AA was studied using a narrow range of concentrations, we next evaluated the tissue's tolerance to higher AA concentrations. Discs prepared from MG2 tomatoes tolerated a 4-h exposure to AA vapors at 9400 μg·g⁻¹, whereas MG4 discs became translucent and water-soaked when exposed to AA at 4000 μg·g⁻¹. However, breaker 'Jackpot' discs prepared from commercially handled tomatoes (pretreated overnight with 100 μL·L⁻¹ C₂H₄) tolerated AA at 5500 μg·g⁻¹, but became translucent and water-soaked at higher AA concentrations. The sensitivity of tomato tissue to AA appeared to increase as it approached the climacteric and then decreased following distinct color formation or C₂H₄ induction.

TISSUE VARIABILITY. Although each experiment contained randomly assorted discs from many fruit of the same maturity classification, atypical results were often observed for one treatment within a concentration series. The inherent variability of biological

material caused results to vary slightly among experiments throughout the season and among cultivars and growing conditions (e.g., greenhouse and field). Since the previous AA levels used in short-term, static exposures that inhibited ripening were close to phytotoxic levels, and to ensure uniform ripening-related responses due to AA absorption, we evaluated long-term exposures to low AA concentrations.

We previously reported that a continuous exposure to low AA concentrations caused profound ripening inhibition, which was fully reversible and which decreased C₂H₄ production in MG3 discs (Beaulieu et al., 1997). When MG4 discs were exposed to a continuous flow of humidified AA vapors, reversible ripening inhibition depended on applied concentration and duration of the exposure (Fig. 4). Ripening was unaffected until day 5 by exposure to AA at 70 to 250 μg·L⁻¹ for 1, 2, or 3 d. The 1-d exposure to all three AA concentrations promoted ripening. As the duration of exposure increased to 2 and 3 d, the inhibitory effect of all concentrations predominated and the start of ripening was delayed for 1, 2, and 4 d by a 3-d exposure to AA at 70, 170, and 250 μg·L⁻¹, respectively. It appears that short exposures to low AA concentrations promote ripening, while longer exposures and higher concen-

Fig. 3. Effect of exposure to acetaldehyde vapors on the subsequent ripening of MG3 and MG4 tomato pericarp discs. Discs were exposed for 4 h to acetaldehyde vapor on a fresh mass basis at 0 to 500 μg·g⁻¹ in a 4-L glass jar. Chromameter a*/b* readings (n=9) were used to calculate lycopene concentrations on a fresh mass basis and 5% LSD values.

trations inhibit ripening. Since the AA concentration in tissue dissipates rapidly, long-term exposures to low AA concentration at 20 °C could be a method to delay ripening before shipping and marketing whole tomato fruit. However, since AA is quite toxic and reactive, EtOH treatment may be more acceptable.

EFFECT OF ADH INHIBITOR. Application of the ADH inhibitor 4-MP eliminated the ripening promotion of EtOH at 1.6 mg·g⁻¹ and the ripening inhibition of EtOH at 3.2 and 6.3 mg·g⁻¹ (Fig. 5). In the presence of 4-MP, all EtOH treatments ripened at a rate similar to the control. These data again indicated that the tissue concentration of AA is crucially involved in ripening-related responses. In previous studies, we showed that increasing applied EtOH levels in conjunction with 4-MP always increased tissue EtOH concentrations, while AA levels were unaffected by the inhibitor (Beaulieu et al., 1997).

Application of 4 mM 4-MP reduced the ripening of control MG3 and MG4 discs by ≈15%, whereas it inhibited the ripening of breaker discs by almost 50%. In the absence of 4-MP, AA at 780 μg·g⁻¹ promoted the ripening of MG3 discs by 7% and inhibited the ripening of MG4 and breaker discs by 5% and 23%, respectively. In the presence of 4-MP, AA at 780 μg·g⁻¹ inhibited the ripening of MG3, MG4 and breaker discs by 30%, 5%, and 19%, respectively. As the maturity of the discs advanced from MG3 to breaker, the ripening of the discs was first promoted and then inhibited by the same concentration of AA. When ADH activity was partially

blocked by 4-MP, the clear effect of maturity on AA activity was lost and AA activity was more effective in only altering the ripening of MG3 discs. The ability of AA to inhibit or promote ripening appears to be related to initial maturity and thereby to the initiation and progression of the respiratory and C₂H₄ climacteric.

EFFECT OF VACUUM TREATMENT. Since AA and EtOH are volatile, their tissue concentration could be modulated by using low pressures to increase their evaporation from treated discs. Application of an 18-kPa vacuum for 24 h immediately after treatment or after 24 h slightly reduced ripening in comparison to control discs (Fig. 6), possibly by reducing endogenous C₂H₄ levels. Exposure to EtOH at 6.3 mg·g⁻¹ for 4 h caused a significant 6-d delay in ripening. Application of vacuum either immediately after the EtOH treatment or after venting for 24 h had a similar effect of significantly reducing the inhibition of ripening by ≈2 d. Exposure to AA at 180 μg·g⁻¹ for 2 d caused a 2-d inhibition of ripening. Ripening inhibition in AA-treated discs was reduced slightly by the 24-h hypobaric treatment. The effect of AA on inhibiting ripening appeared to be immediate and unaffected by removal at low pressure, whereas the effect of EtOH appeared to be protracted since a vacuum could almost abolish the induced ripening inhibition even after 24 h. These data are in accord with our findings that EtOH is slowly metabolized by tomato pericarp tissue to AA, which actually causes the inhibition of ripening.

TISSUE SENSITIVITY. Before ripening, tissue convert relatively low amounts of EtOH to AA under normal physiological condi-

Fig. 4. Effect of extended exposure to acetaldehyde vapors on subsequent ripening of MG3 tomato pericarp discs. Discs were continuously exposed to a flow of air containing acetaldehyde at 0 to 250 μg·L⁻¹ for 1, 2, or 3 d. Arrows indicate the day when tissue was transferred from the acetaldehyde treatments to air. Chromameter a*/b* readings (n = 12) were used to calculate lycopene concentrations on a fresh mass basis and 5% LSD values.

Fig. 5. Effect of an inhibitor of alcohol dehydrogenase on the subsequent ripening of MG3 tomato pericarp discs exposed to ethanol vapors. Discs were treated with 40 μL of water or 50 mM 4-methylpyrazole (4-MP) before exposure for 4-h to ethanol vapor on a fresh mass basis at 0 to 6.3 mg·g⁻¹ in a 4-L glass jar. Chromameter a*/b* readings (n = 18) were used to calculate lycopene concentrations on a fresh mass basis and 5% LSD values.

Fig. 6. Effect of venting and vacuum on the subsequent ripening of tomato pericarp discs exposed to acetaldehyde (AA) or ethanol (EtOH) vapor. Discs were exposed to ethanol vapors on a fresh mass basis at $6.3 \text{ mg} \cdot \text{g}^{-1}$ for 4 h or to acetaldehyde vapors at $180 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ for 2 d. Treatments were followed either immediately or after venting in air for 24 h by an 18-kPa vacuum for 24 h. Chromameter a*/b* readings (12 or 24 replications for AA or EtOH, respectively) were used to calculate lycopene concentrations on a fresh mass basis and 5% LSD values.

tions. For EtOH to have a physiological effect on ripening, it must therefore be present either for long durations or at high concentrations; most literature supports this idea. When EtOH exposures promote ripening, the endogenous levels of AA produced from the applied EtOH are similar to levels found to promote ripening in many studies with various commodities (Hirai et al., 1968; Janes and Frenkel, 1978; Paz et al., 1981; Pesis et al., 1987, 1994). Since the metabolism of exogenously applied AA is rapid in plants (Oppenheim and Castelfranco, 1967) and tomatoes (Beaulieu et al., 1997), prolonged exposures or high concentrations are required to interfere with ripening and decrease in vivo C_2H_4 production (Beaulieu et al., 1997) at the critical point when ripening commences.

Our data suggests that as fruit approach their C_2H_4 climacteric, they were far more sensitive to applied EtOH and AA vapors. The increase of ACC oxidase, which precedes ripening, appears to be a critical control point during maturation. Application of AA to nonclimacteric grape berries inhibited ACC oxidase activity (Pesis and Marinansky, 1992). Ripening of mature-green mango fruit was

not affected by exposure to 0.12% AA vapors for 24 h (Burdon et al., 1994), but ripening was inhibited in fruit exposed to 0.15% to 0.33% AA (Pesis et al., 1994). The increased activity of tomato ADH at the breaker stage (Longhurst et al., 1990) may be a mechanism to reduce internal AA levels that could further affect ripening.

ETHYLENE PRODUCTION. A 4-h static exposure to EtOH at $1.6 \text{ mg} \cdot \text{g}^{-1}$ reduced C_2H_4 production only 2.5% during the exposure, whereas exposure to $6.3 \text{ mg} \cdot \text{g}^{-1}$ reduced production by 25% (Fig. 7). After treatment, discs exposed to $1.6 \text{ mg} \cdot \text{g}^{-1}$ dramatically increased their C_2H_4 production (a false climacteric, which almost masked the climacteric in the control discs), which superseded control levels by 275%, whereas C_2H_4 production from discs exposed to EtOH at $6.3 \text{ mg} \cdot \text{g}^{-1}$ was between 37% and 101% of the control for up to 5 d. Ripening promotion by EtOH at $1.6 \text{ mg} \cdot \text{g}^{-1}$ was likely due to increased endogenous AA levels. In other trials, the same trend was repeated where EtOH-induced ripening promotion reduced C_2H_4 production by $\approx 25\%$ during the 4-h static exposure, while inhibitory EtOH levels reduced C_2H_4 production by 35% to 45% and recovery of C_2H_4 production was significantly delayed (data not shown). Exposure to AA at 1600 or $3100 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ for 4 h substantially reduced C_2H_4 production by 72% and 85%, respectively (Fig. 7); even though the $3100 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ treatment proved to be phytotoxic. After ≈ 1 d, C_2H_4 production recovered in discs exposed to AA at $1600 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ and was similar to control levels, while production by discs exposed to $3100 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ remained low for 5 d before slightly increasing.

Proposed Mechanism

Since the endogenous concentration of AA, rather than EtOH, is the biologically active factor that affects ripening (Beaulieu et al., 1997), the conversion of EtOH to AA is a crucial step in governing whether EtOH promotes or inhibits ripening. If less-than-inhibitory concentrations of EtOH or AA are applied, endogenous AA appears to induce the synthesis of ACC (Mencarelli et al., 1991), promote the nonenzymatic cleavage of ACC (Beaulieu, 1996), or increase ACC synthase activity (E. Pesis, personal communication). Our data concerning short-term exposure to high concentrations, or long-term exposure to low concentrations, of AA are supported by the literature, which indicates that inhibition of ripening is probably due to a reduction in the in vivo activity of ACC oxidase.

Fig. 7. Ethylene production on a fresh mass basis by tomato pericarp discs exposed to acetaldehyde (AA) or ethanol (EtOH) vapors. Discs of 'Dombito' tomato were held overnight after excision to allow wound-induced C_2H_4 to dissipate. Discs were exposed to AA vapors on a fresh mass basis at 1600 to $3100 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ and to EtOH vapors on a fresh mass basis at 1.6 to $6.3 \text{ mg} \cdot \text{g}^{-1}$ for 4 h. Individual data points are the average of three replications.

The concentration of AA in the pericarp disc depends on three factors; the concentration of applied EtOH and its conversion to AA by the tissue, the concentration of applied AA, and the loss of AA either through evaporation or metabolism. Absorption and conversion of EtOH to AA depends on tissue maturity. Tissue AA could alter the activity of ACC oxidase by denaturing the enzyme or stimulating its synthesis, or AA could stimulate the nonenzymatic production of ethylene from ACC. At low AA concentrations, promoted synthesis and activation of ACC oxidase would lead to enhanced conversion of ACC to ethylene and accelerated ripening. At higher AA concentrations, ACC oxidase could be inactivated either through denaturation by AA or by the induction of an inactivating pathway. The difference between the rate of synthesis and inactivation of ACC oxidase would govern its level of activity. When the AA concentration was high, inactivation would equal synthesis, and ethylene production would decline to nonstimulatory levels as ACC oxidase activity declined.

The rise and fall of ACC oxidase activity with increasing tissue AA concentration would therefore explain the ripening-promotive effects of AA at low concentrations and the inhibitory effects at high concentrations. However, another complication could also be affecting ripening. Since ripening is promoted by the biological activity of ethylene, and not just its tissue concentration, any factor that interfered with its action would reduce ethylene promoted ripening. EtOH is just such a factor, and endogenous level of EtOH could modify the biological activity of ethylene endogenously synthesized in response to AA. While this proposed mechanism accommodates all the current data, additional studies of the induction and activity of ACC oxidase are needed to confirm its validity.

Literature Cited

- Abeles, F.B., P.W. Morgan, and M.E. Saltveit, Jr. 1992. Ethylene in plant biology. 2nd ed. Academic Press, San Diego.
- Beaulieu, J.C. 1996. Roles of acetaldehyde and ethanol in inhibition or promotion of tomato fruit ripening. PhD diss., Univ. of California. p. 178.
- Beaulieu, J.C., G. Peiser, and M.E. Saltveit. 1997. Acetaldehyde is a causal agent responsible for ethanol-induced ripening inhibition in tomato fruit. *Plant Physiol.* 113:431-439.
- Blomstrand, R., H. Östling-Wintzell, A. Löf, K. McMartin, B.-R. Tolf, and K.-G. Hedström. 1979. Pyrazoles as inhibitors of alcohol oxidation and as important tools in alcohol research: An approach to therapy against methanol poisoning. *Proc. Natl. Acad. Sci. USA* 76:3499-3503.
- Brecht, J.K. 1995. Physiology of lightly processed fruits and vegetables. *HortScience* 30:18-22.
- Burdon, J.N., S. Dori, E. Lomaniec, R. Marinansky, and E. Pesis. 1994. Effect of prestorage treatments on mango fruit ripening. *Ann. Appl. Biol.* 125:581-587.
- Burdon, J.N., S. Dori, R. Marinansky, and E. Pesis. 1996. Acetaldehyde inhibition of ethylene biosynthesis in mango fruit. *Postharvest Biol. Technol.* 8:153-161.
- Campbell, A.D., M. Huysamer, H.U. Stotz, L.C. Greve, and J.M. Labavitch. 1990. Comparison of ripening processes in intact tomato fruit and excised pericarp discs. *Plant Physiol.* 94:1582-1589.
- Chang, L.A., L.K. Hammett, and D.M. Pharr. 1983. Carbon dioxide effects on ethanol production, pyruvate decarboxylase, and alcohol dehydrogenase activities in anaerobic sweet potato roots. *Plant Physiol.* 71:59-62.
- Davis, P.L. and W.G. Chance, Jr. 1969. Determination of alcohol in citrus juice by gas chromatographic analysis of headspace. *HortScience* 4:117-119.
- Fidler, J.C. 1968. The metabolism of acetaldehyde in plant tissues. *J. Expt. Bot.* 19:41-51.
- Gustafson, F.G. 1934. Production of alcohol and acetaldehyde by tomatoes. *Plant Physiol.* 9:359-367.
- Hewage, K.S., H. Wainwright, and Y. Luo. 1995. Effect of ethanol and acetaldehyde on banana ripening. *J. Hort. Sci.* 70:51-55.
- Hirai, J., N. Hirata, and S. Horiuchi. 1968. Effect of oleification on hastening the maturity of the fig fruit. VI. Respiration and changes in the concentration of metabolic substances in the treated fruits with products in oxidative process of fatty acid such as acetaldehyde or ethylene. *J. Jpn. Soc. Hort. Sci.* 37:20-29. (summary in English).
- Janes, W. and C. Frenkel. 1978. Promotion of softening in pear by acetaldehyde, independent of ethylene action. *J. Amer. Soc. Hort. Sci.* 103:397-400.
- Janes, W.C., C.-K. Chin, and C. Frenkel. 1978. Respiratory upsurge in blueberries and strawberries as influenced by ethylene and acetaldehyde. *Bot. Gaz.* 139:50-52.
- Kelly, M.O. and M.E. Saltveit, Jr. 1988. Effect of endogenously synthesized and exogenously applied ethanol on tomato fruit ripening. *Plant Physiol.* 88:143-147.
- Kimmerer, T.W. and T.T. Kozlowski. 1982. Ethylene, ethane, acetaldehyde, and ethanol production by plants under stress. *Plant Physiol.* 69:840-847.
- Lelback, W.K. 1969. Liver cell necrosis in rats after prolonged ethanol ingestion under the influence of an alcohol-dehydrogenase inhibitor. *Experimentia* 25:816-818.
- Longhurst, T.J., H.F. Tung, and C.J. Brady. 1990. Developmental regulation of the expression of alcohol dehydrogenase in ripening tomato fruits. *J. Food Biochem.* 14:421-433.
- Lurie, S. and E. Pesis. 1992. Effect of acetaldehyde and anaerobiosis as postharvest treatments on the quality of peaches and nectarines. *Postharvest Biol. Tech.* 1:317-326.
- Mencarelli, F., P. Savarese, and M.E. Saltveit, Jr. 1991. Ripening of kiwifruit exposed to ethanol and acetaldehyde vapors. *HortScience* 26:566-569.
- Morris, J.R., D.L. Crawford, and R.W. Buescher. 1979. Effects of acetaldehyde on postharvest quality of mechanically harvested strawberries for processing. *J. Amer. Soc. Hort. Sci.* 104:262-264.
- Oppenheim, A. and P.A. Castelfranco. 1967. An acetaldehyde dehydrogenase from germinating seeds. *Plant Physiol.* 42:125-132.
- Paz, O., H.W. Janes, B.A. Prevost, and C. Frenkel. 1981. Enhancement of fruit sensory quality by post-harvest applications of acetaldehyde and ethanol. *J. Food Sci.* 47:270-273, 276.
- Pesis, E. and I. Avissar. 1989a. The post-harvest quality of orange fruits as affected by pre-storage treatments with acetaldehyde vapour or anaerobic conditions. *J. Hort. Sci.* 64:107-113.
- Pesis, E. and I. Avissar. 1989b. Effect of postharvest application of acetaldehyde vapour on strawberry decay, taste and certain volatiles. *J. Sci. Food Agr.* 52:377-385.
- Pesis, E., I. Avissar, and G. Zauberman. 1987. Effect of acetaldehyde vapors, anaerobic conditions, or ethylene on postharvest quality of feijoa fruits. *HortScience* 22:1135. (Abstr. 703.)
- Pesis, E. and R. Ben-Arie. 1984. Involvement of acetaldehyde and ethanol accumulation during induced deastringency of persimmon fruits. *J. Food Sci.* 49:896-899.
- Pesis, E. and C. Frenkel. 1989. Acetaldehyde vapors influence postharvest quality of table grapes. *HortScience* 24:315-317.
- Pesis, E. and R. Marinansky. 1992. Carbon dioxide and ethylene production by harvested grape berries in response to acetaldehyde and ethanol. *J. Amer. Soc. Hort. Sci.* 117:110-113.
- Pesis, E. and R. Marinansky. 1993. Inhibition of tomato ripening by acetaldehyde vapour or anaerobic conditions prior to storage. *J. Plant Physiol.* 142:717-721.
- Pesis, E., R. Marinansky, I. Rot, and A. Weksler. 1994. Effect of postharvest application of acetaldehyde or a short period of anaerobiosis on fruit senescence. *Proc. Royal Soc. Edinburgh.* 102B:473-478.
- Saltveit, Jr., M.E. 1989. Effects of alcohols and their interaction with ethylene on the ripening of epidermal pericarp discs of tomato fruit. *Plant Physiol.* 90:167-174.
- Saltveit, Jr., M.E. and F. Mencarelli. 1988. Inhibition of ethylene synthesis and action in ripening tomato fruit by ethanol vapors. *J. Amer. Soc. Hort. Sci.* 113:572-576.
- Saltveit, Jr., M.E. and A.R. Sharaf. 1992. Ethanol inhibits ripening of tomato fruit harvested at various degrees of ripeness without affecting subsequent quality. *J. Amer. Soc. Hort. Sci.* 117:793-798.
- Saltveit, Jr., M.E. and S.F. Yang. 1987. Ethylene, p. 367-401. In: A. Crozier (ed.), *The principles and practices of plant hormone analysis*. Academic Press.
- Snedecor, G.W. and W.G. Cochran. 1980. *Statistical methods*, 7th ed. p. 233-234. Iowa State Univ. Press, Ames.