Carbon Dioxide and Ethylene Production by Harvested Grape Berries in Response to Acetaldehyde and Ethanol

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Additional index words. 1-aminocyclopropane-l -carboxylic acid, respiratory quotient, Vitis *vinifera*

Abstract. Application of acetaldehyde (AA) at 90 to 360 mM to intact grape berries *(Vitis vinifera* L. cv. Sultanina and *Vitis vinifera* L. cv. 103) caused an increase in CO_2 production rate and a reduction in ethylene evolution rate. The increase in CO_2 production rate was accompanied by a decrease in juice acidity without any change in the total soluble solids content. Addition of ACC to berry halves dramatically increased ethylene production, which was inhibited by AA. Ethanol, applied at the same concentrations as AA, neither caused a reduction in ethylene evolution nor inhibited the conversion of ACC to ethylene. Chemical name used: 1-aminocyclopropane-1-carboxylic acid (ACC).

It is well established that under reduced O_2 levels below **8%** there is retardation of fruit senescence, generally by inhibition of fruit ripening processes, including respiration and ethylene production (Kader, 1986). The first metabolize formed under anaerobic conditions is AA, which is converted to ethanol by the enzyme alcohol dehydrogenase (Cossins, 1978; Kelly and Saltveit, 1988; Pesis and Avissar, 1989). However, application of AA vapors to various climacteric and nonclimacteric fruits like oranges *[Citrus sinensis* (L.) Osb.] (Fidler, 1968; Pesis and Avissar, 1989), figs *(Ficus carica* L.), blueberries *(Vaccinium corymbosum* L.), and strawberries *(Fragaria × annanassa)* (Janes et al., 1978) caused an increase in the CO_2 production. This increase was accompanied by a decrease in acidity in figs (Hirai et al., 1968) and oranges (Pesis and Avissar, 1989). Janes et al. (1979) found a stimulation of O_2 uptake by potato *(Solanurn tuberosum* L.) tubers treated with AA and ethanol, but related this increase to a stimulation of the cyanide-resistant respiration.

Grape is a nonclimacteric fruit (Biale, 1960) that is harvested preferably after it has reached minimum maturity standards since, after harvest, there is almost no change in quality indices (Nelson et al., 1963). However; AA vapors influence the quality of grapes harvested early in the season by increasing their total soluble solids content (TSS) or decreasing their acidity (Pesis and Frenkel, 1989). In addition, AA vapors have a fungicidal effect on stored grapes, and AA-treated berries have been reported to remain firmer during storage, which may increase their resistance to fungal attack (Avissar et al., 1989). The purpose of this study was to investigate the effects of postharvest application of AA or ethanol on CO_2 and ethylene production by grape berries.

Materials and Methods

Plant material. Experiments were carried out with two grape cultivars grown in Israel: *Vitis vinifera* L. CV. Sultanina (seedless) from the Lakhish region and CV. 103 (seeded) from the Golan Heights region. Grapes were harvested in the mid-late harvesting season, when the berries in both cultivars had a high soluble solids content (20% to 21'%) and low acidity (0.7% to 0.9%). Berries were cut individually from the bunch with their pedicels, rinsed in sterile water to which 0.01% of the antibiotic chloramphenicol had been added, and air-dried in a sterile transfer hood.

Application of AA or ethanol. Experiments were performed with whole or half berries. In the first experiment, eight berries of 'Sultanina' grapes or 10 berries of '103' grapes (40 g) per replicate were weighed and placed in 125-ml sterile Erlenmeyer flasks containing two layers of Whatman no. 1 filter papers. One of four solutions was applied: three AA or ethanol concentrations, 90, 180, or 360 mM, and deionized water as the control. All the solutions contained 0.01% chloramphenicol. Two ml of each of these solutions was placed on the filter papers in each Erlenmeyer flask. Each treatment was replicated four times. The same concentrations of AA and ethanol were applied to enable us to compare their relative toxicity on grape berries.

In the second experiment, berries from '103' were cut along their equator; the distal half, without the seeds, was used for the experiment. Four half-berries (6 g) were placed in 50-ml sterile Erlenmeyer flasks containing two layers of filter paper. The berries were placed on the filter paper with their cut surface down. In each treatment ACC (5 mM) was added in addition to AA or ethanol at concentrations between 18 and 180 mM. Two ml of the various solutions were placed in each Erlenmeyer flask containing 0.01% chloramphenicol. Each treatment consisted of four replicates.

Measurements of AA, ethanol, CO_2, O_2, and ethylene. In both experiments, the flasks were closed with rubber caps for 18 h at 20C, and headspace gas samples were taken for the measurement of CO_2, O_2, ethylene, acetaldehyde, and ethanol by gas chromatographic techniques. The CO_2 and O_2 were detected as described by Pesis and Avissar (1989), and the respiratory quotient (RQ) was calculated by dividing CO_2 production by O_2 uptake for each flask. Ethylene was detected by means of a flame ionization detector provided with an alumina column. AA and ethanol in the headspace were detected according to the method of Davis and Chace (1969).

In the experiments with whole berries, the flasks were ventilated in a sterile hood for 24 h and juice was made from each sample after the gas measurements had been completed. The

Abbreviations: AA, acetaldehyde; RQ, respiratory quotient; TSS, total soluble solids content.

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juice was checked for TSS and for titratable acidity. All data are averages of four replicates per treatment. The data were tested by regression analysis or Duncan's multiple range test, as appropriate.

Results and Discussion

AA caused an increase in the CO_2 production rate in intact grape berries (Fig. 1). In 'Sultanina' there was an increase in the rate of CO_2 production with increasing amounts of AA applied up to 180 mM, after which the rate remained almost constant (Fig. 1). The increase in CO_2 production rate was not accompanied by an increase in O_2 uptake rate. This created an increase in the CO_2:O_2 ratio, calculated as RQ (Fig. 1).

Similar behavior was observed in '103' berries, although there was less absolute increase in CO_2 production with the addition of higher AA concentrations. The initial rate of CO_2 production by this cultivar was higher than that of 'Sultanina'. The highest CO_2 production occurred with 180 mM AA (Fig. 1). There was a gradual, slight decrease in O_2 consumption by '103', which led to an increase in the RQ (Fig. 1). The levels of O_2 were ≈ 16% to 17% after 18 h of incubation in all treatments. Addition of ethanol at the same concentrations as AA to berries from both cultivars did not lead to significant differences in CO_2 production rate or O_2 uptake rate (data not shown).

The rate of CO_2 production also increased during application of AA in other nonclimacteric fruits, such as citrus (Fidler, 1968; Pesis and Avissar, 1989), strawberries, and blueberries (Janes et al., 1978). However, in apple, a climacteric fruit, application of AA did not cause such an increase (Fidler, 1968). He suggested that the additional CO_2 produced in AA-treated

oranges was derived from the oxidation of AA to CO_2. In grape berries, the increase in CO_2 production rate was not accompanied by an increase in O_2 uptake rate, which caused an increase in the RQ (Fig. 1). In seed tissue, higher RQ values usually indicate an impaired activity of the mitochondria (Pesis and Ng, 1984).

The increase in CO_2 production was accompanied in both cultivars by a significant reduction in juice acidity and an increase in pH (Table 1), which was measured on the same berries that were previously tested for CO_2 and O_2 exchange. The changes in TSS content were not significant (Table 1).

The reduction in acidity in individual grape berries treated with AA solutions was also found in other fruits. In figs injected with AA solution on the tree, there was a dramatic decrease in acidity accompanied by an increase in TSS content (Hirai et al., 1968). In whole oranges treated with AA vapors, there was a reduction in acidity, an increase in pH, and no change in TSS content of the juice (Pesis and Avissar, 1989). Clusters of grapes picked early in the season and treated with AA vapors were less acidic and had a higher TSS content than controls (Pesis and Frenkel, 1989). Possibly, AA may be oxidized to acetyl-CoA (Cossins, 1978; Fidler, 1968) and, thus, lead to a higher CO_2 production rate via the TCA cycle. Alternatively, AA may increase the production of some decarboxylases, which would account for the increase in CO_2 evolution and lead to a reduction in the total acidity. In previous work, we showed that in grapes picked early in the season (with low sugar content) and after AA application, there was an increase in the TSS content that could be related to gluconeogenesis (Pesis and Frenkel, 1989). However, in the current experiment, we used grape berries with a high TSS content (20% to 21%), and AA treatments probably did not affect gluconeogenesis. In pears *(Pyrus communis* L.), tomatoes, and blueberries *(V. corymbosum* L.), AA led to an increase in the reducing and total sugar concentrations (Paz et al., 1982).

Application of even the lowest concentration of AA (90 mM) to whole grape berries of both cultivars substantially inhibited ethylene evolution rate. The higher concentration induced a slight decrease with an increase in AA concentrations applied (Fig. 2). The untreated 'Sultanina' berries had a much higher rate of ethylene production than '103'. At the same concentrations used for AA, ethanol did not cause a decrease in ethylene production (data not shown), indicating that AA maybe more toxic than ethanol to grape berries.

The inhibition of ethylene production by application of AA has not been previously demonstrated to our knowledge. However, there are several papers about the inhibition of ethylene
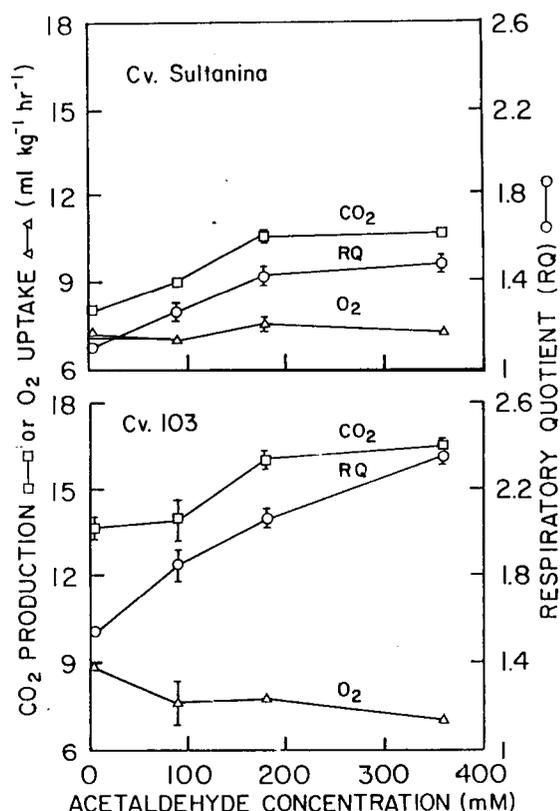

Fig. 1. CO_2 production, O_2 uptake, and the RQ by whole 'Suhanina' and '103' grape berries in response to treatment with various concentrations of AA after 18 h of incubation at 20C. Error bars represent SE of the mean where this value exceeds the size of the symbols.

Table 1. Effects of several concentrations of AA solutions on juice acidity, pH, and TSS content of 'Sultanina' (seedless) and '103' (seeded) grape berries.

AA (mM)	Sultanina			103		
	TSS (%)	pH	Acid (%)	TSS (%)	pH	Acid (%)
0	20.8	2.96	0.67	21.3	2.90	0.94
90	20.6	3.13	0.67	21.2	2.95	0.85
180	20.3	3.14	0.59	21.7	2.98	0.83
360	20.4	3.19	0.58	21.5	3.09	0.79
r	0.457[NS]	0.535*	0.635*	0.347[NS]	0.562*	0.602*
SE from regression	0.5491	0.0888	0.0512	0.3681	0.0665	0.0580

r = Correlation coefficient with applied AA.
[NS],* Nonsignificant or significant at $P = 0.01$ (df = 14).

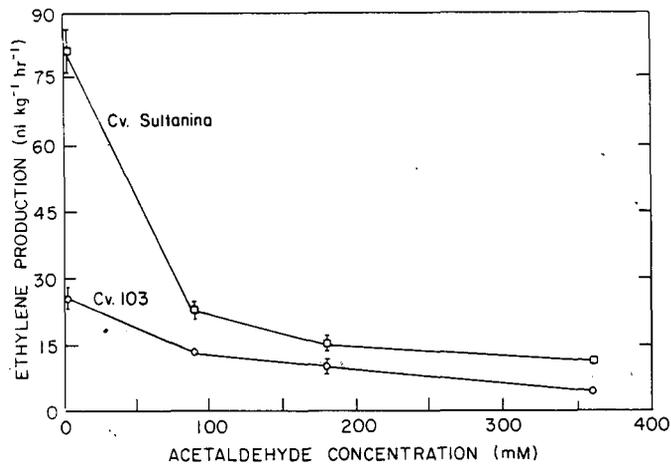J. Amer. Soc. Hort. Sci. 117(1):110-113. 1992.

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Fig. 2. Ethylene production by whole 'Sultanina' and '103' grape berries in response to treatment with various concentrations of AA after 18 h of incubation at 20C. Error bars represent SE of the mean where this value exceeds the size of the symbols.
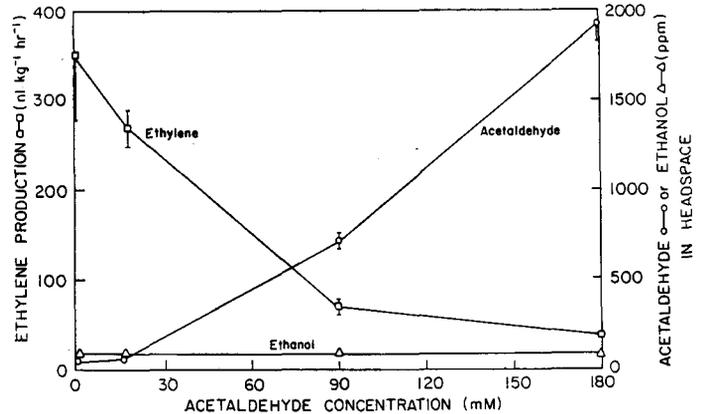

Fig. 3. Ethylene production by halves of '103' grape berries and concentrations of AA and ethanol vapors in response to treatment with various concentrations of AA and ACC (5 mM) after 18 h of incubation at 20C. Error bars represent SE of the mean where this value exceeds the size of the symbols.

Table 2. Effect of AA (180 mM) and ethanol (180 mM) application in solutions on ACC (5 mM) conversion to ethylene in halves of '103' grape berries after 18 h of incubation at 20C.

ACC	Ethylene production (nl·kg⁻¹·h⁻¹)		
	Water	AA	Ethanol
	28 a[z]	22 a	21 a
+	320 a	58 b	334 a

'Mean separation in rows. by Duncan's multiple range test, $P = 0.01$.

production by ethanol. In climacteric tissues, 2% ethanol in the holding solution inhibited climacteric ethylene synthesis and delayed senescence of carnation *(Dianthus caryophyllus* L.) flowers (Heins, 1980). Ethanol was also found to inhibit ethylene and lycopene production in tomato *(Lycopersicum esculentum* Mill.) slices (Saltveit and Mencarelli, 1988). However, it is possible that, in both cases, the reactive compound is AA since ethanol can reconverted into AA through alcohol dehydrogenase (AIM.), which exists incarnation flowers and tomato slices. Cossins (1978) showed that the first metabolize in ethanol use by plant tissue is AA. Recently, Perata and Alpi (1991) showed that ethanol was toxic to carrot cells only after it was converted into AA since the addition of an ADH inhibitor (4-methylpyr-azole) prevented the toxicity of ethanol.

The production of ethylene by untreated whole '103' berries was very minor (Fig. 2); therefore, we chose to study the effect of ACC application on the ethylene production of this cultivar. In halved berries of '103' the level of ethylene produced without addition of ACC was very low (Table 2). Apparently, very little wound ethylene is produced by this cultivar, unlike in tomato, which produced much higher amounts of wound ethylene (Kende and Boiler, 1981). This divergence could be due to the difference between climacteric and nonclimacteric fruit, and the fact that we used halved, not sliced, fruit. The low ethylene production by the halved berries enabled us to apply ACC without first inhibiting ethylene production, as was done in tomato (Saltveit and Mencarelli, 1988).

Without the addition of ACC, 180 mM AA or ethanol did not significantly affect the ethylene production rate of halved '103' berries (Table 2). This result is in contrast to the one for whole berries where 180 mM AA substantially reduced the ethylene

evolution rate (Fig. 2). Application of 5 mM ACC to halved berries increased ethylene production ≈ 10-fold over the untreated berries (Table 2). This stimulation of ethylene production by ACC was similar to that in climacteric fruits, such as apple (Mansour et al., 1986) and tomato (Saltveit and Mencarelli, 1988).

Addition of 180 mM AA, but not ethanol, to the incubation medium caused a significant reduction in ethylene production relative to ACC added alone (Table 2). Moreover, application of lower concentrations of AA (18 to 90 mM) to halved berries of '103' that also were treated with ACC (5 mM) decreased ethylene production by the berries. Eighty percent of the ethylene production was inhibited by 90 mM AA, while doubling the concentration of AA (180 mM) caused additional inhibition of only 8%.

For AA-treated halves of '103' grape berries, AA in the headspace atmosphere was positively related with the amount applied in the solutions, while the amount of ethanol in the headspace was minimal at all AA concentrations (Fig. 3). This result probably indicates that AA, not ethanol, inhibits the conversion of ACC to ethylene in grape berries; it may also indicate that the conversion of AA to ethanol by halved berries is very low. In previous work on grapes, we showed that application of AA vapors to clusters of grapes led to the production of ethanol that was positively correlated with the amount of AA applied (Pesis and Frenkel, 1989). It is possible, therefore, that the injury caused by cutting the berries in half, or the application of AA in solutions, reduced the grapes' ability to convert AA to ethanol by alcohol dehydrogenase, possibly because AA inactivates this enzyme.

In conclusion, it was shown that AA, but not ethanol, caused inhibition of ethylene production but not of CO_2 evolution or O_2 uptake. The mode of action of AA on ethylene inhibition remains to be studied.

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