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Methyl Bromide Inhibits Ripening and Ethylene Production in Tomato (*Lycopersicon esculentum* Mill.) Fruit

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Abstract. Tomato fruit ripening and ethylene production were inhibited following treatment with methyl bromide (MB). Methyl bromide significantly delayed ripening initiation in mature-green (MG) fruit and retarded the rate of ripening of turning (T) fruit as measured by color development and flesh softening. Treatment with MB caused an initial transient burst of ethylene production, but the subsequent ripening-associated increase in ethylene was delayed. Ethylene treatment partially overcame MB inhibition in MG fruit but had no affect on T fruit. The inhibition of ethylene production by MB appears to be due to lack of formation of 1-aminocyclopropane-1-carboxylic acid (ACC) in MG fruit, whereas in T fruit lack of conversion of ACC to ethylene is indicated. A key feature of MB inhibition of ripening in tomato appears to be reduced sensitivity to ethylene.

The fumigant methyl bromide (MB) is widely used in agriculture as an insecticide and nematicide (Thompson 1975). It is the only fumigant currently recommended in the U.S. for disinfestation of fruits that serve as hosts to the various fruit fly species (USDA 1979). MB is an effective quarantine fumigant, yet its use is not without problems. One disadvantage is the severe phytotoxic response exhibited by some fruit types following exposure to MB levels required for insect mortality (Knight et al. 1983). Other fruit types, including tomato, show little or no fumigant-induced injury, but they may instead respond by exhibiting significantly attenuated rates of ripening. The inhibitory effects of MB on tomato fruit ripening have been known since the work of Mackie (1938) and Jones (1940), who reported a delay in color development in tomato fruit subjected to MB fumigation. The extent of ripening inhibition appears to be dependent on MB dosage and fruit maturity, with green tomatoes more sensitive to MB than fruit of more advanced ripening classes (Lipton et al. 1982).

The mechanism by which MB inhibits fruit ripening has not previously been investigated. Although delayed color development is the most frequently noted

effect of MB fumigation, it is apparent that other ripening processes are similarly affected. Other reported MB effects include inhibition of softening in various stone fruits (Claypool and Vines 1956, Harvey et al. 1982), inhibition of catalase activity in papaya (Jones 1940), and reduced respiration rates during tomato fruit ripening (Knott and Claypool 1940). Current theories of ripening and ripening initiation place great emphasis on the regulatory role of ethylene (Rhodes 1980). Ethylene synthesis (Yang and Hoffman 1984) and perhaps the status of ethylene receptor sites (Smith and Hall 1984) could be important points of regulation. It seemed possible that MB might function as a growth regulator in inhibiting ripening initiation. To determine the mechanism of MB inhibition of fruit ripening, we treated preclimacteric and ripening tomatoes with MB and studied the ripening changes and ethylene production of the fruit. In addition, we investigated the effect of MB on ethylene action by applying exogenous ethylene to MB-treated fruit.

Materials and Methods

Tomatoes used in these experiments were either commercially harvested and packed or harvested locally over two seasons and transported to Gainesville, Florida, for treatment. Fumigation with MB was carried out in cooperation with R. Brown and J. C. Nickerson, Florida Department of Agriculture and Consumer Services. Cultivars tested included Sunny, FTE-12, Hayslip, and Flora-Dade. Following holding overnight at 20°C, tomatoes were allowed to warm to room temperature (28°C) and then treated with MB at 32 g/m^3 for 3.5 h (USDA 1979). Control fruit were held at the same temperature. Following MB treatment, fruit were sorted for uniform size and color development. Only fruit with no trace of external pink or tannish-yellow color were used in the mature-green (MG) treatments. Fruit designated as turning (T) showed color development over 10–30% of the surface. Fruit were placed at 20°C and treated with 50 μl/l ethylene for 2.5 days via a flow-through system. Fruit (10–12 per treatment) selected for daily monitoring of ethylene production, color, and firmness were placed at 25°C in 500-ml glass jars and allowed to ripen. Additional fruit were reserved for measurement of ACC levels during ripening (5 fruit per treatment at each sampling date). All experiments described here were repeated at least twice over the 2-year period; representative data are shown.

To determine the ethylene production rates of individual fruit, jars were sealed for 1–2 h and the concentration of ethylene in a 1-ml sample of the headspace was determined by gas chromatography. Ethylene was measured daily except for immediately prior to and 2 h following treatment with MB and ethylene. Fruit surface color was measured with a HunterLab tristimulus color difference meter using *L*, *a*, and *b* modes. The instrument was calibrated with a standard white reference plate ($L = 94.9$, $a = 1.2$, $b = 2.2$), and fruit color was measured at two opposite equatorial locations. Values for *a* and *b*, respectively, were averaged and the *a*/*b* ratio calculated. As tomatoes ripen, *a* values rise and *b* values decline, indicating increasing red (lycopene) and yellow (carotene) color, respectively. Fruit firmness was determined by measuring deformation in millimeters caused by a 16-mm convex probe positioned equatorially over a locule and applied with a force of 9.8 N for 5 s. Five fruit per treatment were selected periodically and frozen at −25°C until used for determination of

Table 1. Firmness and color of FTE-12, Sunny, and Hayslip tomatoes fumigated at two color stages with 32 g MB/m³ for 2.5 h at 28°C. Larger values indicate softer fruit and greater color development.

Treatment	Days at 25°C	MG stage		T stage	
		Firmness (mm deformation)	Color (*a*/*b* ratio)	Firmness (mm deformation)	Color (*a*/*b* ratio)
Control	4	0.87	0.50	2.25	2.12
MB		0.68	0.08	2.00	1.71
Control	10	1.58	1.69	—	—
MB		1.26	0.80	—	—

Interactions between cultivars and fumigation treatment were not significant, so average values for the three cultivars are presented. Differences between pairs of numbers are all significant at 1% level by F test.

Table 2. Effect of MB and ethylene on ripening (color development) of MG (Sunny) tomatoes.

Treatment	Days to breaker	Days to red ripe	Days from breaker to red ripe
Air	6.7 ± 1.4	13.8 ± 0.8	7.1
MB	10.7 ± 1.0	19.0 ± 0.8	8.3
Ethylene	3.9 ± 0.3	11.9 ± 0.5	8.0
MB + ethylene	9.5 ± 1.0	17.1 ± 1.3	7.6

Breaker fruit were determined by the first appearance of tannish-yellow or pink surface color; red ripe fruit were postclimacteric and uniformly red. Values ± SE.

ACC concentration. Approximately 5–10 g of skin and outer pericarp tissue were homogenized with a Polytron homogenizer in trichloroacetic acid (2:1, v:w). ACC levels were measured by the method of Lizada and Yang (1979).

Results

Treatment with MB consistently retarded the ripening of all four cultivars of tomatoes at either the MG or T stage. Initial experiments showed that firmness was retained and color development was retarded in MB-treated fruit (Table 1). When MG fruit were treated with MB, the onset of ripening was delayed by at least 3 days in all tests (Table 2). Ethylene treatment (50 μl/l) significantly decreased the days to reach the breaker stage compared to air control fruit but had little effect on MB-treated MG fruit (Table 2). Ethylene had no effect on T fruit, with or without MB treatment (data not shown). Thus ethylene treatment at 50 μl/l did not negate the MB-associated inhibition of ripening. Table 2 also illustrates that the time for fruit to develop from the breaker to the red ripe stage did not differ appreciably among the treatments. That is, once ripening of MB-treated fruit was initiated, it proceeded at a rate comparable to that observed for control fruit.

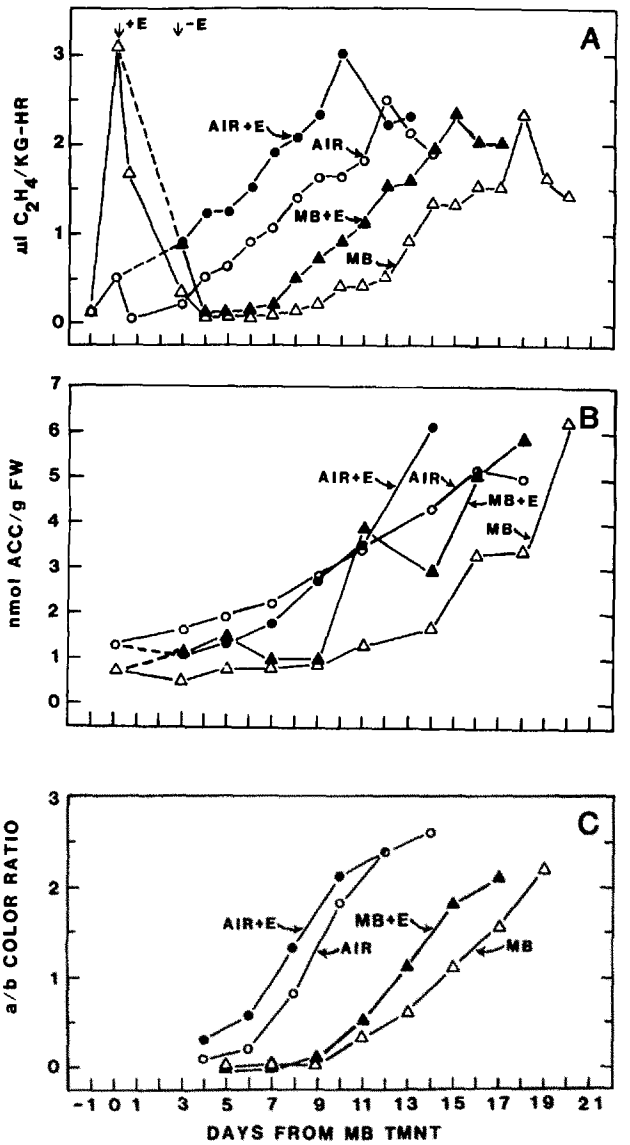

Fig. 1. Ethylene production (A), ACC levels (B), and *a/b* color ratios (C) of MG (Sunny) tomatoes (○, air control; ●, air + C_2H_4; △, methyl bromide; ▲, methyl bromide + C_2H_4). Measurements of ethylene production rate and color were repeated with individual fruits. ACC determinations were made on samples of identically treated fruit that were frozen and stored at −25°C after various intervals.

The effect of MB on ethylene production of MG fruit is shown in Fig. 1A. The initial response to MB was a greater than 30-fold increase in ethylene production followed by a return to prefumigation levels over several days. The onset of climacteric ethylene production was delayed by about 6 days in MB-treated fruit. Treatment with 50 μl/l ethylene following MB exposure resulted in only about a 4-day delay. Control fruit exposed to ethylene initiated ethylene production without any lag period. The changes in ACC levels in relation to the changes in ethylene production during ripening are shown in Fig. 1B. The increases in ACC corresponded to the onset of ethylene production in the air

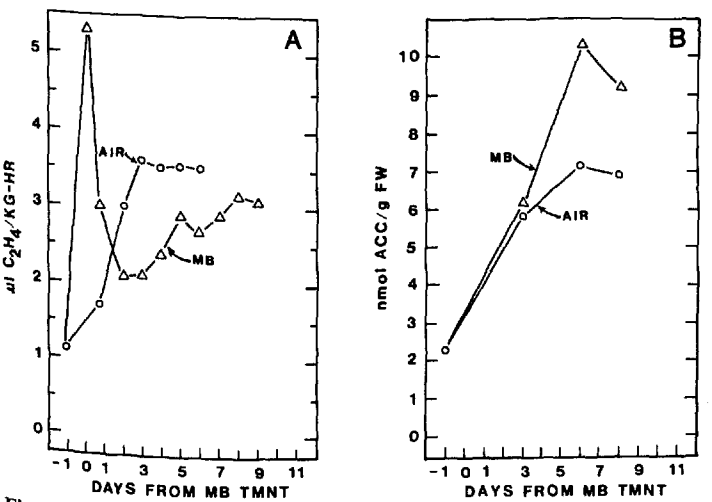

Fig. 2. Ethylene production (A) and ACC levels (B) of Flora-Dade tomatoes treated at the T stage (○, air control; △, methyl bromide). Procedures as in Fig. 1.

and air plus ethylene treatments, but appeared to lag behind the increase in ethylene in MB and MB plus ethylene treated fruit. Color development (Fig. 1C) followed a similar pattern as ethylene, but significant color development was not apparent until ethylene production exceeded about 1 μl/kg-h.

When MB was applied to fruit that had already initiated ripening, there was an initial fivefold increase in ethylene production, after which ethylene declined for 2 or 3 days before again increasing (Fig. 2A). Control fruit ethylene production rose in a typical climacteric fashion, reaching the maximum rate on day 3, whereas MB-treated fruit did not reach their maximum rate until day 8. In contrast to MG fruit, the ACC level in treated T fruit was equal to or greater than the control level whereas ethylene production was inhibited (Fig. 2B).

Discussion

At least two possible mechanisms have been proposed regarding the action of MB in biological systems (McKenry 1981). The first involves reaction of MB with a nucleophilic site—e.g., OH, SH, or NH_2 groups—in a vital enzyme or other protein system. The second involves oxidation by MB of the iron complexes of heme proteins such as the cytochromes, peroxidase, or catalase. Either of these mechanisms could cause general phytotoxic effects in plant tissues leading to cell death. However, despite a large burst of (apparently) stress-induced ethylene production, we observed no physical evidence of tissue injury in MB-treated tomatoes. Furthermore, following the MB-induced delay, ripening proceeded normally in treated fruit. Thus, the pattern of the inhibitory response observed in these studies suggests a reversible process, perhaps related to diffusion of MB from lipid-rich regions of the cell, where it is known to accumulate in animal systems (Cremlyn 1981), or to turnover of an affected enzyme or other protein.

The action of ethylene in fruit ripening may be regulated by changes in its rate of synthesis or by the sensitivity of the tissue to ethylene. The autocatalytic increase in ethylene production during fruit ripening may be considered an example of ethylene action, since ethylene stimulates ethylene production by the fruit. Both formation of ACC and conversion of ACC to ethylene may be stimulated by ethylene (Yang and Hoffman 1984). The effect of MB on MG fruit was to delay ethylene production and ACC accumulation; ethylene production in T fruit was also inhibited by MB, but ACC levels were higher than in control fruit. The first case suggests inhibition by MB of ACC synthase (Kende and Boller 1981), the enzyme responsible for ACC formation; the second case appears to involve lowered conversion of ACC to ethylene in MB-treated fruit. In neither case, however, did exposure of the MB-treated fruit to a high level of ethylene, well above the saturating dose for ethylene responses (Abeles 1973), restore the normal ripening rate. Thus, the reduced ability of these fruit to respond to ethylene appears to be responsible for their reduced rates of ethylene production and ripening. This idea supports an essential role for ethylene not only in the initiation of fruit ripening but also in the progression of ripening in initiated fruit.

Sisler (1980) has recently presented evidence for an ethylene-binding component in mung bean that appears to be a membrane protein. In light of the lipophilic nature of MB, we propose that it is absorbed by the lipids of the cell membranes where it can interact with ethylene-binding sites or otherwise disrupt the expression of ethylene's effects. Desorption of MB or synthesis of new receptors could eventually overcome the MB effect. In addition, it is possible that MB may exert a direct effect on the enzymes of ethylene biosynthesis or other enzyme systems. Confirmation of these possibilities must await a better understanding of the structures and/or mechanisms of, on the one hand, ethylene-binding sites and ethylene action, and, on the other hand, ACC synthase and the ethylene-forming enzyme.

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