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Changes in Tomato Fruit Ripening Caused by Ethylene Dibromide Fumigation[1]

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Abstract. **Ethylene dibromide (EDB) fumigation of fruit of tomato (*Lycopersicon esculentum* Mill.) reduced red color development in the outer pericarp, although the inner tissues remained unaffected at EDB doses as high as 35 g/m^3. Carotene accumulation was enhanced by EDB at 4 g/m^3, but at higher doses the carotene content of the tomato pericarp was reduced. Skin puncture force was reduced in green fruit fumigated at 4 g/m^3, but not in breaker or pink fruit; higher skin puncture forces were recorded at higher doses for the three fruit maturities tested, EDB stimulated the respiration of preclimacteric fruit, but fruit fumigated just prior to the climacteric showed a normal respiration peak, although a 4 g/m^3 treatment resulted in partial climacteric respiratory rise.**

Fumigation with 1,2-dibromoethane (ethylene dibromide, EDB) effectively disinfests fruit against several fruit flies, with treatment schedules well established for Oriental and Mediterranean fruit flies (3, 12, 8, 14). The EDB concn recommended for these insects, however, is inadequate against *Dacus tryoni* (Frogg.) (10, 16), a fruit fly indigenous to north eastern Australia.

Development of a disinfestation treatment for tomato fruit against *D. tryoni* has been hindered by the high incidence of injury following fumigation with EDB. Pratt, Baughn and Getty (15) found that tomatoes were generally intolerant of EDB fumigation; an 8 mg/liter treatment retarded ripening, with green fruit being more affected than pink fruit.

Methyl bromide, an alternative, insecticidal fumigant, similarly retards tomato ripening and color development (1). Furthermore, the respiration of mature green tomato fruit increases immediately after fumigation with methyl bromide (9), but declines over 3-5 days to a level similar to that of the preclimacteric stage of control fruit; respiration then rises to normal climacteric levels. Similarly, fumigation with EDB causes an immediate rise in respiration of deciduous fruits (4) and bananas (6), and also retards the later stages of banana ripening.

In this paper we report the effect of EDB fumigation on visual color, carotene accumulation, firmness, respiration and ethylene production of tomato fruit. The fruit were fumigated at the green, breaker and red maturity stages.

Materials and Methods

'Grosse Lisse' and 'Indian River' tomatoes were selected from local growers and color graded into the maturity classes of green and breaker, according to the standard of the USDA 'Color Classification Requirements in Tomatoes' (2). 'Rutgers' fruit, for the respiration and ethylene production studies, was obtained from W. B. McGlasson of CSIRO Division of Food Research. These fruit were harvested from hydroponically grown plants 35 days after anthesis, and dipped in a fungicide suspension containing 600 mg/liter dichloran plus 300 mg/liter benomyl to reduce postharvest decay.

Fumigation procedure. Fruit was fumigated with EDB for 2 hr at 20°C in a galvanized iron, water-sealed chamber of 283 liter capacity (16). All fruit was temperature conditioned at 20° for 18 hr prior to fumigation. After treatment, the fruit was aerated for 2 hr in the fumigation chamber, with the lid removed and the circulation fan operating. Single fruits, for the respiration and ethylene production studies, were fumigated in a 2.5 liter glass jar fitted with a screw top lid.

Visual assessment of EDB injury. Samples of 10 green and 10 breaker fruit were fumigated with a range of EDB doses and stored for 6 days at 20°C. Fruits were then individually examined under standard, white fluorescent light and scored for appearance as follows: 1 = green color; 2 = pale pink; 3 = dark pink; 4 = red color, with small colorless areas; and 5 = red color with no colorless areas. For each sample of 10 fruits a weighted average color score was calculated, and the mean fruit scores resulting from each of the fumigation doses compared.

Fruit firmness. Twenty green, breaker and pink fruit were fumigated with a range of EDB doses and stored for 6 days at 20°C. The force (gram wt) required to puncture the skin of each fruit was then measured with a Mercer dial gauge pressure tester, fitted with a 3 mm diam, rounded head. Puncture tests were carried out at one point on each fruit, in the equatorial region of the fruit, external to a locule. Mean skin puncture force was calculated for each fruit sample.

Respiration and ethylene production. Green fruit harvested 35 days after anthesis were individually weighed and placed in separate glass respiration jars, ventilated by a calibrated flow of humidified air (about 1 liter/hr) at 20°C. On the day of harvest, and 4 and 7 days after harvest, individual fruits were fumigated with EDB doses of 0, 4, 16 and 32 mg/liter. Production of carbon dioxide and ethylene was monitored daily until 5 – 6 days after the climacteric peak of the control fruit as previously described (13).

Effect of EDB on carotene accumulation. Green and breaker fruit was randomly allocated to 62 treatment units, with 20 fruit of each maturity in each treatment unit. Five units were fumigated at each of the following doses: 0, 2, 4, 6, 8, 12, 16, 20, 25, 30, 40 and 50 mg/liter. Following fumigation and aeration, all fruit was held at 20°C for 6 days and then assayed for carotene content. The remaining 2 treatment units of each maturity class were not fumigated, but assayed for carotene content on the day of fumigation.

All fruit in each treatment unit were homogenized, and samples of the homogenate (50 g) were extracted with cold acetone, and this extract partitioned against petroleum ether (bp 60–80°C). The extract was washed free of acetone with 10% NaCl solution and dried by filtering through anhydrous Na_2SO_4. The carotene content (μg/g dry wt) of the tissue was determined as either lycopene or β-carotene using the $E^{1\%}_{1\,cm}$

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values of 3450 and 2505 respectively (5).

Results

EDB injury and carotene accumulation. Color development in tomato fruit fumigated with EDB was generally reduced and uneven. A fumigation dose of 4 mg/liter or less had only a marginal effect on fruit appearance, whereas fruit fumigated at higher EDB doses were mostly pale in color with several small, colorless areas. The mean score for fruit color declined with increasing EDB dose (Fig. 1). The response of 'Indian River' fruit to EDB fumigation followed the same pattern as that for 'Grosse Lisse' fruit, although the actual scores for the two cultivars were different ($P < 5\%$). The reduction in color development was restricted to the outer pericarp. This effect was particularly noticeable in green fruit fumigated at the higher EDB doses, where the outer pericarp of such fruit had little red color. The pericarp of green and breaker tomato fruit fumigated with 4 mg EDB/liter had a higher carotene content than that of unfumigated fruit (Table 1). The carotene content of the fruit pericarp 6 days after fumigation with higher EDB doses was lowered with increasing dose; at 40 mg EDB/liter (green) and 25 mg EDB/liter (breaker) the carotene content of the pericarp 6 days after treatment was similar to that at the time of fumigation of green (287 ± 53 μg/g dry wt, β-carotene) and breaker fruit (572 ± 87 μg/g dry wt, lycopene).

EDB effect on fruit softening. The skin puncture force of fruit fumigated with 4 mg EDB/liter was less than that of the untreated fruit (Fig. 2), although this difference was only significant ($P < 5\%$) with green fruit. The skin puncture forces of pink and breaker fruit fumigated with 12 and 20 mg EDB/liter were greater than those for untreated fruit ($P < 5\%$); for green fruit fumigated with these EDB doses the skin puncture force was similar to that of the untreated fruit.

Respiration and ethylene production. The respiration of all fruit fumigated with EDB on the day of harvest (Fig. 3A) or 4 days after harvest (Fig. 3B) rose immediately whereas only the 4 mg/liter dose increased respiration of fruit fumigated after 7 days at 20°C. With fruit fumigated on the day of harvest, 4 mg EDB/liter had little effect on the pattern of respiration (Fig. 3A). Although the initial respiration rate of these fruit following fumigation was approximately twice that of the untreated control, the respiration recovered within 2 to 3 days to the control level. Respiration rate of fruit fumigated with 16 and 32 mg/liter EDB, however, remained well above

Table 1. Carotene content of the pericarp of tomato fruit fumigated with EDB. Fruit was treated at the green and breaker stages with EDB and held for 6 days at 20°C.

EDB dose (mg/liter)	Carotene content (μg/g dry wt)[z]	
	Green	Breaker
0	611 ab	1186 op
2	626 ab	1186 op
4	681 a	1508 m
6	548 bcd	1466 mn
8	569 bc	1300 no
12	557 bcd	1042 p
16	497 cd	1075 p
20	486 cd	785 q
25	490 cd	634 qr
30	463 d	605 r
40	308 e	605 qr
50	275 e	689 qr

[z]Mean separation by Duncan's multiple range test, 5% level.

that of the control fruit, and did not show a normal climacteric; carbon dioxide production from these fruits was still rising when the experiment was terminated.

The pattern of ethylene production of these fruit fumigated on the day of the harvest (Fig. 3A) was similar to that of their respiration, with the 16 and 32 mg EDB/liter-treated fruit producing an increasing amount of ethylene when the experiment was stopped. After the 32 mg/liter fumigation, the rate of ethylene production was reduced, and that from the fruit treated with 16 mg/liter was considerably higher than that of the control fruit.

Fumigation of fruit held 4 days after harvest at 20°C with 4 and 16 mg/liter EDB caused an immediate rise in respiration (Fig. 3B) followed by a decline over 3 to 4 days to normal levels. The 32 mg/liter treatment caused the greatest rise in respiration, which remained high during the course of the experiment, without a climacteric peak being observed. Once again, the 16 mg/liter fumigation caused the greatest rise in ethylene production. The pattern of ethylene production following a 4 mg/liter treatment was similar to that of the untreated fruit, although the rate of production was higher.

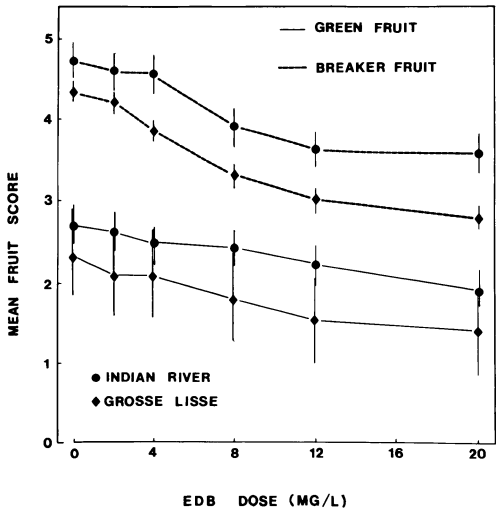

Fig. 1. Effect of EDB on mean fruit score of 'Grosse Lisse' and 'Indian River' tomato fruit. Green and breaker fruits were fumigated with EDB doses of 0, 2, 4, 8, 12 and 20 mg/liter and scored, after 6 days storage at 20°C, on the basis of skin color and EDB injury.

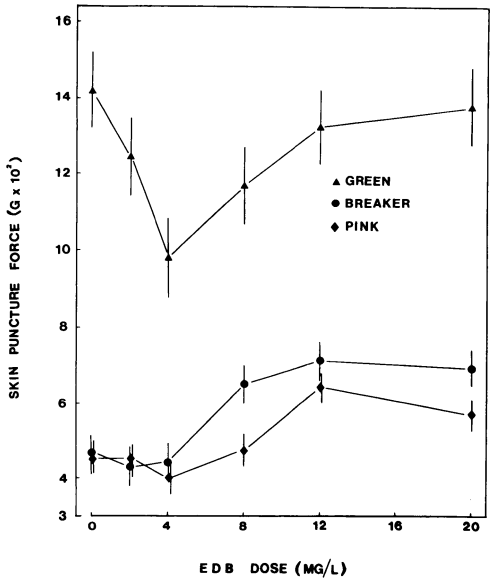

Fig. 2. Effect of EDB on skin puncture force of 'Grosse Lisse' tomato fruit. Fruit was fumigated with EDB doses of 0, 2, 4, 8, 12 and 20 mg/liter and after 6 days storage at 20°C, skin puncture force was determined as gram wt (G) on the equatorial region of each fruit.

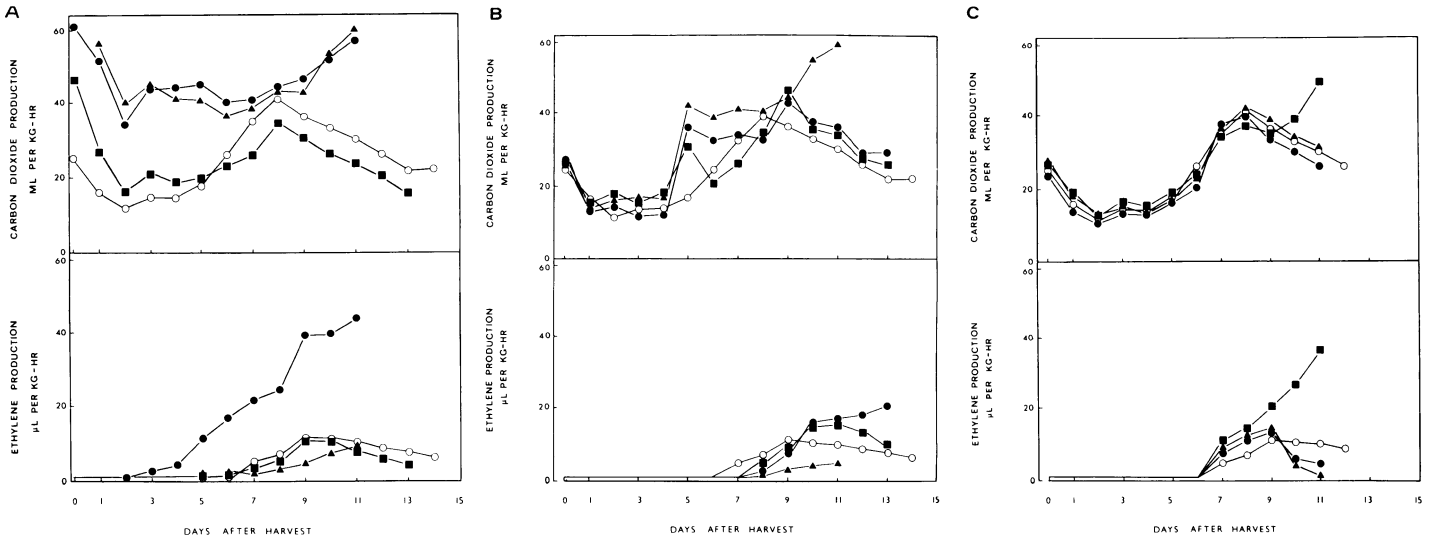

Fig. 3A, B, C. Effect of EDB on respiration and ethylene production by 'Rutgers' tomato fruit. The data presented are representative of 4 fruits treated with each EDB dose. Fruit was fumigated with EDB doses of (○—○)0, (■—■)4, (●—●)16 and (▲—▲)32 mg/liter. A) Fruit was harvested 35 days after anthesis and fumigated on the day of harvest. B) Fruit was harvested 35 days after anthesis and fumigated 4 days after harvest. C) Fruit was harvested 35 days after anthesis and fumigated 7 days after harvest.

With fruit fumigated after 7 days at 20°C, little effect on respiration was observed immediately after fumigation (Fig. 3C), and both the 16 and 32 mg EDB/liter-treated fruit showed a normal climacteric peak. A 4 mg/liter dose, however, caused a rise in respiration some 3 days later, and the respiration rate of these fruit was still rising at the conclusion of the experiment.

This same pattern was once again reflected in the production of ethylene by these fruits.

Discussion

A direct relationship between color development and EDB dose is apparent, with the higher EDB doses resulting in reduced fruit color, and thus a lower fruit score. The nature of this relationship, which is constant for the two cultivars tested, suggests that EDB at low doses does not act as a "switch" by which pigment syntheis can be turned off, but rather that an increase in EDB dose results in a gradual decline in fruit color. The EDB injury to tomato fruit is restricted to the outer pericarp, with little or no color reduction apparent in the internal tissue of the fumigated fruit. Since the fruit score is an assessment of the external appearance only, this normal internal color was not reflected in the mean fruit scores.

Fumigation with 4 mg EDB/liter stimulates carotene synthesis in tomato fruit, while concn greater than 8 mg EDB/liter inhibit carotene accumulation. Fumigation of green fruit with 40 mg EDB/liter, and of breaker fruit with 25 mg/liter, results in virtually no further carotene synthesis; after fumigation at these doses the carotene level 6 days after treatment is similar to the level found at the time of fumigation.

Fruit respiration is stimulated by EDB fumigation, with a 4 mg/liter EDB dose having less effect than higher doses, except for fruit fumigated at the peak of the climacteric, where higher EDB doses have no effect on respiration; however, respiration is stimulated by 4 mg EDB/liter.

EDB is readily absorbed by the fumigated fruit, especially the lipid fraction of such fruit (20); it is therefore reasonable to expect that EDB is preferentially absorbed onto membranes and other lipid-based constituents of the cell. Heuser (1973, personal communication) considers EDB to be a strong alkylating agent and capable of combining with amino acids and proteins, as well as nucleic acids. Such alkylation may well cause changes in the conformation and activity of intracellular membranes and their associated enzyme systems. Inhibition of membrane-associated enzymes could affect carotene synthesis, respiration and the enzymic degradation of pectic substances, since all these processes involve enzymes associated with cell membranes.

Lewis (11) suggested that methyl bromide, a similar alkylating agent to EDB, reacts preferentially, under physiological conditions with sulphydryl groups on proteins. Since phytoene synthetase is thought to possess a sulphydryl group at the active site (7), such alkylation of this enzyme would cause inhibition of all carotene synthesis.

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Response of Cole Crops to Combinations of Herbicides and Insecticides [1]

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Abstract. **Over a 6-year period (1969-1974) the efficacy of 3 insecticides and 24 herbicides and their interactions in combination were investigated when applied to field-seeded broccoli (*Brassica oleracea* L. Italica group), cabbage (Capitata group) and cauliflower (Botrytis group). Of these, broccoli was the most susceptible to injury. Of 212 herbicide-insecticide combinations, 26 caused phytotoxic symptoms in broccoli, 20 in cabbage and 8 in cauliflower. The insecticides, thionazin, fensulfothion and carbofuran, were each involved in 1 or more phytotoxic combinations in each of the 3 crops. Ten herbicides were involved in phytotoxic reactions: alachlor, aziprotryn, benefin, CDEC, chlorpropham, cycloate, prometryne, propachlor, prynachlor and PP493. Root maggot damage was reduced markedly by the insecticides. Carbofuran allowed less damage than either fensulfothion or thionazin. None of the herbicides showed any insecticidal properties, and some decreased the effectiveness of the insecticides.**

In the production of precision-drilled cole crops, insecticides for root maggot control are usually applied at drilling. Herbicides are normally applied with the insecticides or within a short period, but combined pesticide treatments may be phytotoxic (3, 4, 5). This report covers a 6-year (1969-1974) investigation to examine the actions of 24 herbicides and 3 insecticides and their interactions when applied in combination on field-seeded broccoli, cabbage and cauliflower.

Materials and Methods

'Northwest Waltham 29' broccoli; 'Golden Acre' cabbage and 'Snowball Y' cauliflower were seeded in a silt loam with a multiplegear V-belt seeder in 1969-71 and with a tractor mounted Stanhay Mark II precision seeder in 1972-74. The experimental design was a split plot, randomized block, with 4 replicates. In 1969-71 each plot consisted of one 24-m row for each crop. The plots were divided into 6-m sub-plots, 1 for each insecticide and a control. In 1972-74, since only 2 insecticides were used, the rows were 18 m long.

Herbicides (Tables 1 and 2) were selected on the basis of previous unpublished work, or reported effectiveness for weed control in brassicas. They were applied under pressure at 0.10 kg/cm^2 as preplant soil incorporated (ppi), preemergence (preE), or postemergence (postE) sprays. The ppi treatments were rotovated in immediately after application; the preE treatments were applied before emergence of weeds and crop; the postE treatments were applied when most of the weeds were at the first true-leaf stage.

The insecticides thionazin (Zinophos), fensulfothion (Dasanit) and carbofuran (Furadan) were selected for control of cabbage maggot, *Hylemya brassicae* (Bouché) because of proven efficacy (1, 2). In 1969-71 all herbicides were tested in combination with each of the 3 insecticides. In 1972-74 they were combined only with carbofuran and fensulfothion following withdrawal of thionazin by the manufacturer. The insecticides were applied as granules at 2 g a.i./10m of row in a 10-cm band over the row immediately after seeding and raked gently into the soil, or incorporated by the "bow wave" method produced by the coulter of the Stanhay seeder. A supplementary drench at 2 g a.i./liter per 10 m to wet the plants and 7.5 cm of soil on each side of the row was applied 28 days after seeding The drench was applied under pressure with a hand sprayer. Sprinkler irrigation was applied when necessary.

The compatibility of the pesticide combinations was assessed by their effect on seedling emergence, plant ht, yield, and maggot damage compared to the control plants. Seedling emergence was determined by counting the emergent seedlings at the first true-leaf stage. Plant ht was measured at thinning time, about 28 days after seeding. Yield included the total of several harvests of produce from each sub-plot. Estimates of maggot damage were made on 10 roots/sub-plot as follows: 0 = none, 1 = light, 2 = moderate, 3 = severe, and 4 = very severe. This index is expressed as % damage; 100% would indicate 10 roots with very severe damage.

Results and Discussion

Ppi treatments. Two deleterious herbicide-insecticide combinations were identified in the ppi treatments in broccoli, 3 in cabbage and 1 in cauliflower (Table 1). DCPA + CDEC with thionazin reduced seedling emergence in broccoli in 1970 and in cauliflower in 1969, and stunted initial growth of cauliflower. In the 1968 trial (4) DCPA + CDEC with thionazin reduced plant stand in cabbage and cauliflower but not in broccoli. DCPA + CDEC with fensulfothion caused stunting

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