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Ethylene Dibromide Fumigation of Citrus Fruit to Control the Mediterranean Fruit Fly, *Ceratitis capitata* (Wied.)[1]

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Abstract. **Grapefruit, orange, and lemon fruits were fumigated with ethylene dibromide (EDB) at 12 and 14 g/m^3 for 2 hr exposures against eggs, larvae, and pupae of the Mediterranean fruit fly. Grapefruit also was fumigated at 16 g/m^3 for 2 and 2.5 hr exposures. EDB sorption was determined in an empty chamber and when the chamber was loaded with fruit. At EDB dosages of 12 and 14 g/m^3, a complete kill of eggs, larvae, and pupae was obtained by fumigating artificially infested oranges and lemons. With grapefruit, complete kill of eggs and pupae and high mortality of larvae were obtained at those dosages. When the dosage was increased to 16 g/m^3 and exposure prolonged to 2.5 hr a 100% kill of larvae was obtained. All citrus fruits were tolerant to dosages used and no peel injury occurred during subsequent 1 month storage and 2 weeks under shelf-life conditions. The rate of EDB residue desorption from various citrus fruits after fumigation was determined. The amounts of inorganic bromide residue resulting from fumigation were below safety limits.**

Many importing countries require citrus fruit to be properly treated either by cold or by fumigation with EDB (4, 13). This is to ensure freedom from viable stages of fruit fly eggs, larvae, and pupae, so as to prevent their introduction into uninfested areas.

Several works deal with citrus fruit tolerance to fumigation with EDB against the Oriental fruit fly, (*Dacus dorsalis* Hendel), and with the presence of bromide residues resulting from treatment (11, 12). Fumigations against the Mediterranean fruit fly involves citrus fruit with very few larvae, and results in high survival (9). More recent work has focused on fruit tolerance and inorganic bromide residues (1, 8, 14).

The main objective of this study was to include all aspects of EDB fumigation, to determine the relationship between conditions of treatment, physiological response of the fruit, toxicity to various developing stages of the Mediterranean fruit fly, and residues of EDB and inorganic bromide in the fruit.

Materials and Methods

Artificial fruit infestation. 'Marsh' grapefruits, 'Shamouti' oranges, and 'Eureka' lemon fruits were selected from a single orchard. These fruits were subjected to the customary export packinghouse treatment, treated with 0.5% water solution of sodium orthophenylphenate, and waxed with a natural emulsion wax which contained 0.3% Thiabendazole [TBZ, 2-(4'thiazolyl) benzimidazole]. To study the effect of the EDB on different stages of fly development, fruits were infested artificially with medfly eggs (10) on successive days prior to fumigation and placed in an incubation room at 29 ± 1°C and 85–90% relative humidity (RH) for larval development. An average of 200–250 eggs were injected into every fruit. When fumigated, infested fruits contained larvae at different instars. One day prior to fumigation, 1 bruce box of fruits was injected with eggs, allowing data to be obtained on the effect of EDB on eggs.

Before fumigation fruits were wrapped in nonimpregnated biphenyl tissues, cooled to 16–21°C, and packed in standard bruce boxes (31 x 31 x 48 cm). Pupae from different pupation dates were also put separately in organdine bags within bruce boxes, in between the fruits. The boxes were then placed at different locations in the fumigation chamber.

Fumigation treatments. Procedures used have been previously reported elsewhere (2). The total load of the chamber was constant and consisted of 69 bruce boxes, always arranged in 13 stacks of 5 plus 1 stack of 4. These were placed on a false floor 20 cm high, so that 76% of the 11.1 m^3 total volume of the fumigation chamber was occupied. Each experiment involved boxes with infested fruits, with specially selected noninfested fruits (for the determination of peel injury after fumigation and cold storage), and with nonselected fruits to make up the load. On the basis of percentage load, fruit temperature and chamber volume, the amount of EDB was calculated to give desired dosages. A measured amount of the liquid EDB fumigant was volatilized in a pyrex beaker over an electric hotplate which was located under a blower in the center of the fumigation chamber. The vapor was mixed with the circulating air by the blower.

EDB concn in the atmosphere of the chamber was measured during the fumigation period by an interferometer specially assembled by the Technion, Technological Institute of Israel. Atmospheric samples were drawn from the chamber at a rate of 70 liters/hr with the aid of a small pump through copper tubing and a flowmeter into the interferometer.

At the end of the 2 or 2.5 hr exposure the fumigant was discharged through a fresh air delivery and exhaust duct system. Fumigated boxes were aerated for 48 hr at ca. 20°C, the infested fruit was kept at 24° for 10–14 days and then examined for fly survival. Boxes with nonifested fruit were placed in storage for peel injury determination.

Mortality check. On the day of the fumigation 10 nonfumigated fruits from each injection date were opened to determine the larval instar at the time of fumigation and natural larval mortality. There was a nonsignificant number of dead larvae in the nonfumigated fruit.

Ten to 14 days after fumigation, each fruit was opened and checked for larval mortality. All larvae (dead ones usually were brown by that time) were counted and recorded. Surviving larvae were transferred within a piece of fruit into a plastic cup covered with silk cloth and placed in an incubator (27°C,

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70–75% RH) for further observations on pupation and fly emergence. The fumigated pupae were placed in an incubator (27°, 70–75% RH) for 28 days, then counted and checked for fly development.

Fruit injury. To study the effect of EDB on fruit peel, fumigated and nonfumigated (control) grapefruit, orange, and lemon fruits at various sizes were examined for peel injury after storage at 10, 6, and 13°C, respectively, and 80–85% RH for 2 or 4 weeks and after 2 additional weeks under shelf-life conditions at ca. 20°C.

Two tests were designed to study the effect of EDB fumigation, at the highest dosage, on degreened grapefruit peel. Degreened grapefruit was treated with 20 ppm ethylene gas for 30 hr, at 24–26°C and 88–90% RH and then subjected to the customary export packinghouse treatment as mentioned previously.

EDB residues. Residues in the various fruits were determined by the method of Bielorai and Alumot (6). Inorganic bromide content was also determined after the storage period.

Results and Discussion

Efficiency of Fumigation. No difference in the EDB concn were detected at the 9 sampling points within the fumigation chamber. Only ca. 15% of the initial gas concn was sorbed in the empty chamber after 2 hr fumigation period. The greater loss of EDB in the presence of 69 bruce boxes of grapefruits was due to sorption by the load (Fig. 1). Total EDB sorption at the end of the fumigation period was 44.1, 60.7, and 69.3% at 12, 14, and 16 g/m^3, respectively. Similar results were obtained while fumigating oranges and lemons together. At all dosages, EDB concn declined to 0 within a few minutes after the exposure period.

Mortality of eggs, larvae and pupae. In tests with eggs exposed to 12 g EDB/m^3, 4 live larvae were found in grapefruit and 3 in lemon (Table 1). All larvae from grapefruit pupated but none from lemon. However, adults did not emerge. At 14 g EDB/m^3, 2 larvae were found in grapefruit. They developed abnormally and did not pupate. At exposures of grapefruit to 16 g/m^3 for 2 or 2.5 hr, no eggs developed into larvae following fumigation. Egg survival decreased as the concn of EDB increased. The eggs are more easily and effectively killed within oranges than within grapefruit or lemon.

With grapefruit, larvae survived at 12 and 14 g EDB/m^3 and subsequently developed to pupae and adult flies. Grapefruit requires higher dosage (16 g/m^3) and longer exposure time (2.5 hr) to achieve a 100% kill (Table 2). When oranges were fumigated, some larvae survived at dosages of 12 and 14 g/m^3, but none developed to an adult fruit fly. Complete kill of larvae also was obtained in lemon at these dosages.

In these tests 65,694, 80,831, and 55,635 pupae were fumigated at dosages of 12, 14, and 16 g EDB/m^3, respectively for 2 hr, and 32,856 pupae at 16 g EDB/m^3 and 2.5 hr. At all 3 dosages and with all citrus fruit there was a complete kill. Pupae were readily killed with EDB. Naturally, pupae could not be found inside the fruit, but pupae may have originated from an infested fruit after packing. In this case, they were exposed directly to the fumigant which did not have to penetrate into the fruit to be effective. This is probably the reason for the complete kill.

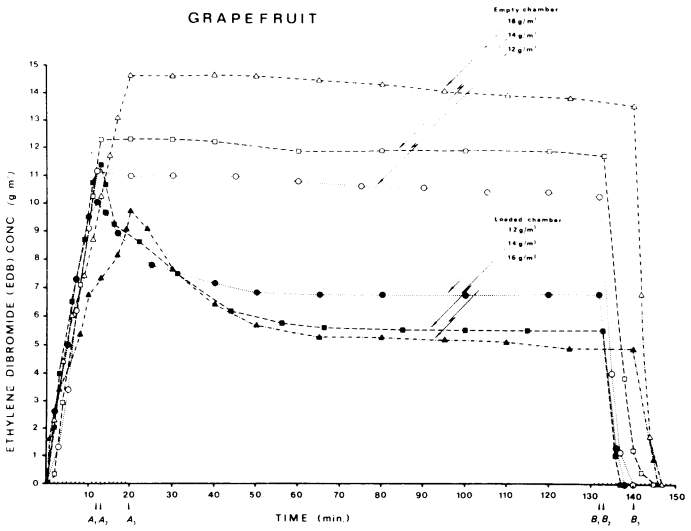

Fig. 1. Atmospheric concentrations of EDB as measured by an interferometer during fumigations at different dosages in the fruit fumigation chamber when empty and when loaded. A_1, A_2, A_3 – Time of complete volatilization of the EDB. B_1, B_2, B_3 – Termination of the fumigation period and beginning of aeration.

An effective quarantine treatment must control all stages of the fruit fly, i.e., eggs, larvae and pupae. Each test included these stages and % mortality was determined from the total population. The results show that the same concn of EDB produced a complete kill of all stages of the insect in oranges and lemons. At dosages of 12, 14, and 16 g EDB/m^3 with grapefruit and 2 hr duration of fumigation, there was 99.9967, 99.9966, and 99.9982% mortality respectively, but an exposure of 2.5 hr at 16 g EDB/m^3 was required to produce a 100% mortality.

Some countries, including the U.S., require that quarantine treatments against fruit flies be based on a procedure developed by Baker (5). He recommended a treatment that would produce 99.9968% mortality. Such a treatment would allow 32 survivors from a population of 1,000,000.

Table 1. Efficiency of grapefruit, orange, and lemon fruit fumigation with EDB against eggs of the Mediterranean fruit fly.

EDB dosage (g/m^3)	Duration of fumigation (hr)	Fruit temp. (°C)	No. of tests	No. of infested fruits	Estimated no. of eggs	No. of live larvae	No. of pupated larvae	No. of emerging adult flies
				Grapefruit				
12	2	16-19	2	97	17,270	4	4	0
14	2	21	2	96	25,920	2	0	0
16	2	16-20	5	256	53,760	0	0	0
	2.5	16-18	5	231	66,280	0	0	0
				Orange				
12	2	16-19	2	107	23,980	0	0	0
14	2	19-21	3	177	55,320	0	0	0
				Lemon				
12	2	16-19	3	205	64,790	3	0	0
14	2	19-21	3	236	73,060	0	0	0

Table 2. Efficiency of grapefruit, orange, and lemon fruit fumigation with EDB against larvae of the Mediterranean fruit fly.

EDB dosage (g/m^3)	Duration of fumigation (hr)	Fruit temp. (°C)	No. of tests	No. of infested fruits	No. larvae		No. of pupated larvae	No. of emerging adult flies
					Dead	Surviving		
				Grapefruit				
12	2	16-19	2	1,701	27,478	12	9	2
14	2	21	2	1,884	38,027	8	7	3
16	2	16-20	5	3,891	102,884	18	12	4
	2.5	16-18	5	6,153	80,195	0	0	0
				Orange				
12	2	16-19	2	2,550	21,851	12	0	0
14	2	19-21	3	2,977	17,757	6	2	0
				Lemon				
12	2	16-19	3	3,296	31,968	0	0	0
14	2	19-21	3	2,487	16,938	0	0	0

Results reported here are not only in agreement with recommendations of the U.S. Dept. of Agr. (4), but show that a reduced dosage of EDB from 14 to 12 g/m^3 at 15.6–20.6°C and 50–80% fruit load in the chamber will provide the required kill in grapefruit, orange, and lemon fruit.

Fruit tolerance. No peel injury occurred in 832, 1,712, and 4,336 grapefruits fumigated at dosages of 12, 14, and 16 g EDB/m^3 for 2 hr, respectively. There was no sign of injury to 5,624 grapefruits even fumigated at a dosage of 16 g EDB/m^3 for 2.5 hr. This is in contrast to previous results (8) in which peel injuries occurred at lower EDB dosages. TBZ used in our tests for decay control perhaps prevented peel injuries (7).

Fumigating 1,275 and 2,625 oranges, and 2,118 and 3,330 lemons at 12 and 14 g EDB/m^3 for 2 hr, respectively did not cause peel injury. Also, 4 weeks of cold storage did not cause pitting in 4,638, 1,595, and 2,480 nonfumigated grapefruits, oranges and lemons respectively, nor in the fumigated fruits. Moreover, no peel injury to the fruit occurred after a subsequent 2 weeks under shelf-life conditions at ca. 20°C.

The tests showed that degreened grapefruit was slightly more sensitive to EDB fumigation than nondegreened fruit. Only 0.5% of the 6,120 degreened fruits showed slight and moderate peel injury following fumigation and cold storage for 1 month. Perhaps, some degree of peel injury might occur when degreened grapefruit are fumigated at the beginning of the season.

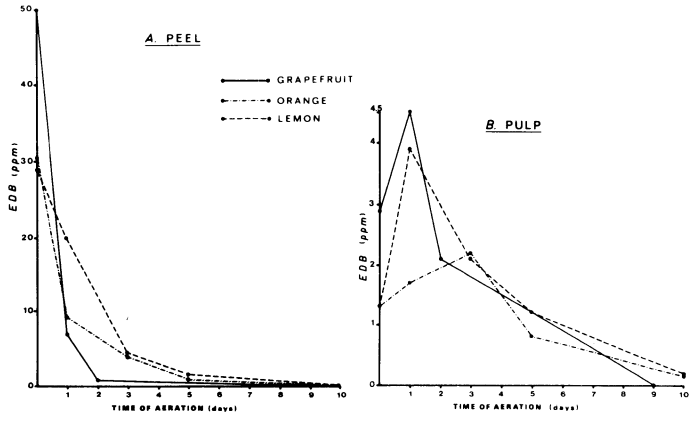

Fig. 2. Residues of EDB (ppm) in the peel (A) and pulp (B) of grapefruit fumigated with 16 g/m^3 of EDB for 2.5 hr and orange and lemon fruits fumigated with 14 g/m^3 of EDB for 2 hr. Following the fumigation period the fruit was subsequently aerated at room temperature (ca. 20-22°C).

EBD residues. Residues in the fruit peel decreased rapidly following fumigation (Fig. 2-A). Although, grapefruit was fumigated at a higher dosage (16 g/m^3), exposed for a longer time (2.5 hr), and had a higher initial EDB content, residues in the peel decreased at a much faster rate than in orange or lemon peel.

EDB present in the peel was higher than in the pulp (Fig. 2). The greatest quantity of EDB in the pulp was found in grapefruit and lemon 1 day after fumigation, and 3 days in oranges and then started to decrease rapidly (Fig. 2-B). The different EDB diffusion rate might be related to the difference in the peel and pulp of these fruits. Evidently, the EDB diffuses from the peel both to the outside air and into the pulp (12). When the content in the peel is already too low to cause an increase in the pulp content, the content of the pulp decreases, also.

Table 3. Inorganic bromide residues in grapefruit, orange, and lemon fruits fumigated with different doses of EDB.

EDB dosage (g/m^3)	Duration of fumigation (hr)	Inorganic bromide (ppm)	
		Control	Fumigated
		Grapefruit	
12	2	1.5	4.1
14	2	1.3	4.1
16	2	2.1	5.8
	2.5	1.2	4.4
		Orange	
12	2	1.6	4.9
14	2	1.4	6.2
		Lemon	
12	2	2.4	6.0
14	2	2.4	6.1

The residue content of inorganic bromide in fumigated fruit was higher than in the nonfumigated control (Table 3). However, there was little difference between the various EDB dosages used. The inorganic bromide content of the nonfumigated control lemons was higher than in grapefruit and oranges. This probably accounted for the higher residues found in the fumigated lemons. From the results in Table 3 it was evident that, at all dosages and exposures, total inorganic bromide residues in grapefruit, orange, and lemon fruits were below the official tolerance of 10 ppm listed for citrus fruit (3).

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Inheritance of Eight Characters in Intra- and Interspecific Crosses Among Five *Carica* Species[1]

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Abstract. **The inheritance of 8 monogenically controlled plant, fruit, and seed characters in *Carica* species is reported. The gene for red stem is dominant to that for green stem and the gene for red petiole is dominant to that for green stem and the gene for red petiole is dominant to that for green petiole. Genes for white and purple-blush flower colors are dominant to those for pale yellow; while the gene for red skin color of ripe fruit is dominant to that for yellow. However, the gene for red skin color is not dominant to that for orange skin color; the heterozygote has pink-skinned fruits. The gene for ridging on the fruit (carpel fusion lines) is dominant to that for wide groove, which in turn is dominant to that for narrow groove. Spiny vs. non-spiny seed coat produces an intermediate F_1, indicating no dominance. The gene for succulent fruit pulp is dominant to that for dry pulp. The gene for bushy branching is dominant to that for sparse branching.**

Genetic studies of vegetative characters in the Caricaceae have largely been confined to cultivars of *Carica papaya* L., the only species of commercial importance. These investigations were motivated by attempts to discover sex-linked characters which might allow the identification of sexes in the early seedling stages. Recent studies on compatibility and interspecific hybridization among several *Carica* species (7, 8) provided an opportunity to determine the genetics of 8 characters.

Usually, color of organs of plants has been reported to be controlled by a single gene pair. Variations in color intensities usually were not investigated, but were attributed to modifying gene action. In a biochemical survey of factors determining flower color, Scott-Moncrief (10) showed that many different gene types were involved in flower color variations, some exhibiting independent action with their effects being purely additive, while others expressed interactions of a complex nature.

Pigmented stem and petiole generally show monogenic inheritance with pigmented stems and petioles dominant over green in Spanish clover (*Desmodium sandwicense* E. Mey) (9), carrot (*Daucus carota* L.) (1), and guava (*Psidium guajava* L.) (6), although exceptions have been reported in jute (*Corchorus capsularis* L.) (4), okra (*Hibiscus esculentus* L.) (3), and sesame (*Sesamum indicum* L.) (2).

In *Carica papaya* the gene for yellow flower color was dominant to that for white (5). This gene was sex-linked. In the 'Kapoho Solo' purple-tinged flower color was also found to be a sex-linked character limited to hermaphrodites. Female flowers were all white (Nakasone, unpublished data). However, sex vs. flower color linkages offer no practical value inasmuch as sex can also be determined by floral morphology at flowering.

Hofmyer (5) found also that purple stem and petiole in *C. papaya* were dominant over green stem and petiole. The observed differences in intensity of the purple color were not analyzed but modifying factors affecting intensity were suggested.

Inheritance studies of skin color of ripe fruits have not been reported in *Carica,* largely due to the lack of color variations in *C. papaya.* There is a mutant yellow cultivar (with yellow leaves and fruits) in which the gene for yellow color of both leaves and skin of immature fruits is recessive to that of normal green

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