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LOW DOSE ETHYLENE DIBROMIDE FUMIGATION FOR QUARANTINE CONTROL OF CARIBBEAN FRUIT FLY IN GRAPEFRUIT[1]

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Abstract. **In an attempt to reduce ethylene dibromide (EDB) residue in fumigated grapefruit following the quarantine treatment, a series of experiments varying certain controllable parameters of the fumigations was performed. Time of fumigation and applied dose are the 2 parameters most commonly controlled; temperature, although controllable, is generally set by nature and the environment. Combinations of temperatures between 50 and 80°F; times between 0.25 and 4.0 hr; and EDB dose rates of between 0.25 and 8.0 oz/1000 ft³ airspace were applied to fruit fly infested white Marsh grapefruit. Insect mortality and EDB residue data indicate that a treatment combination of 4 oz EDB/1000 ft³ for 2.5 hr at a fruit and chamber temperature of 75°F may be used to achieve probit 9 mortality of insects infesting grapefruit and will result in a low concentration of EDB in the fruit following fumigation. Statistical analyses indicate that temperature was not a highly significant factor in the fumigation process in the temperature range tested (P = 0.23).**

'Marsh' white grapefruit [*Citrus paradisi* (Macf.)] and certain other subtropical fruits grown in Florida are susceptible to infestation with immature stages of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (8, 10). Since 1974, grapefruit shipped to Japan has been required to have an acceptable quarantine fumigation to prevent the introduction of *A. suspensa* into that country. California, Texas, Arizona, and Hawaii also require fumigation of suspected host fruits before they will allow them to be brought into their states. Although ethylene dibromide (EDB) has been the fumigant of choice for controlling most tropical fruit flies and until recently the only nonphytotoxic means of quarantine treatment for the fruit, the Environmental Protection Agency (EPA) banned its use on citrus fruits for consumption within the United States as of September 1, 1984 (4). Citrus fruit for export outside the territorial United States may still be fumigated during certain months (4). The quarantine treatment until 1984 has consisted of an applied EDB dose of 8 or more ounces per 1000 ft³ airspace, depending on load factor and temperature, for a 2-hr time period (11).

As early as 1958, McPhail (6) projected a probit 9 mortality rate when grapefruit infested with the Mexican fruit fly, *A. ludens* (Loew), were treated with EDB at a 4 oz/1000 ft³ rate for 2 hr. Tests in 1976 by Burditt and von Windeguth (2) suggested a dose rate of at least 6.5 oz/1000 ft³ if the 95% lower confidence limits was used as the minimum acceptable dose. Preliminary low dose tests by von Windeguth and King (9) indicated that by lengthening the fumigation time, an applied dose of 4 oz/1000 ft³ might give acceptable quarantine control of the infesting insect. If the grapefruit importing country is still willing to accept EDB as a quarantine treatment, this lower level of chemical in the fruit would both cut down on worker exposure during shipment and consumer ingestion in the importing country.

Experimental Design

The "central composite rotatable design for 3 factors" (or independent variables) as described in Cochran and Cox (3) calls for 20 separate experiments, but we added 23 more tests to the areas in the experimental region that looked sparse. In the design, the 5 levels of each factor are coded −1.68, −1, 0, 1, and 1.68. For each factor, the coded values −1.68 and 1.68 correspond to the lowest and highest levels. The 3 intermediate levels are calculated by proportional parts. Thus, the actual EDB treatment dose values were 0.25, 1.4, 3.9, 6.3, and 8.0 oz/1000 ft³ airspace. (The 3.9 oz center dose was due to a mathematical error when the 0 value was derived by subtracting the lowest from the highest dose and dividing by 2; it should have been 4.1 oz). Chamber and fruit temperatures were 50, 56, 65, 74, and 80°F, and fumigant exposure times were 0.25, 1.01, 2.13, 3.24, and 4 hr. For this series of tests, load factor (% of chamber filled with fruit) was not considered a variable since in the State of Florida all semi-trailer fumigation chambers are <25% full when loaded.

Materials and Methods

Grapefruit were infested by placing them in an outdoor screened infestation cage which contained ca. 100,000 gravid female Caribbean fruit flies. The grapefruit were laid out on metal hardware cloth racks and exposed to the flies for one week. After removal from the infestation cage and cleaning, the fruit was reboxed in 4/5 bu fiberboard boxes and placed in a temperature controlled walk-in refrigerator for at least 24 hr to allow all the infested fruit to reach the desired fumigation temperature. Time of fumigation and EDB applied dose were randomly selected for each test for that week. It was not possible to randomly select temperatures within a given week because of the need to precool or heat fruit and chamber to the same temperature. Generally, only one temperature variable was selected for all 6 tests during a given week.

Following the fumigation period and a 1 hr aeration period (the aeration period was 1 hr regardless of the length of the fumigation), the boxed fruit were removed from the 50-ft³ chamber. At this time 4 fruit were selected randomly from the fumigated load (1 fruit each from 4 different boxes) for residue analyses. The remaining fumigated fruit (250 to 280 fruit/test) were placed in screened bioassay towers as described by Burditt and von Windeguth (1). The fumigated fruit and controls were retained for a minimum of 4 weeks following the fumigation. The larval collection boxes filled with sand were screened weekly to collect pupae that had developed from larvae surviving the fumigation. For the purposes of these tests, a pupae recovered from a fumigated lot of fruit is considered a survivor even though no adult emerges from the pupari-

[1]Mention of trade names, proprietary products, or commercial companies does not imply endorsement of that product by the U.S. Department of Agriculture over similar materials.

um. Because of environmental conditions in the sand (liquification by-products of rotten grapefruit), non-emergence of an adult could be due to the post fumigation environment and not the fumigation.

EDB residues were determined individually on each of the 4 grapefruit removed from each test. The method used for residue determination involved steam distillation from a benzene-water mixture for separation and cleanup. A gas chromatograph equipped with a Nickel-63 electron capture detector was used to determine the concentration of EDB present in the samples (5).

The confirmatory phase of the tests was performed in a 9000 ft^3 semi-trailer fumigation chamber. The boxed infested fruit was placed at a central location in a 1000-box dummy load (styrofoam balls) within the trailer. Fumigation was done as described by Burditt and von Windeguth in 1976 (2).

Results and Discussion

The mean EDB residue and percent mortality data from the 43 tests are presented in Table 1. The Generalized Linear Models procedure of the SAS computer program (7) was used to obtain the multiple linear regression of percent mortality and of EDB residue on the logarithms of EDB dose, time of fumigation, and temperature (of the fruit and fumigation chamber area). As expected, the effects of EDB dose and time were significant (at the 0.01% and 0.8% levels, respectively). We were surprised that temperature was not significant ($P = 0.23$ or significant only at the 23% level). Perhaps temperature is not quite as important as we believed when considered as a function in an EDB fumigation. The coefficient of determination (r^2 value) for the multiple regression was 0.7941.

The equation for the regression of the percent mortality on the natural logarithms of EDB dose, time of fumigation, and temperature is given below:

$$\% \text{ mortality} = -17.7029 + 26.3601 (\text{Ln dose}) + 17.3150 (\text{Ln temp}) + 7.4915 (\text{Ln time})$$

If the values of any 2 of the 3 factors (dose, temperature and time) are fixed, we can calculate the value of the third factor that will give any predicted percent mortality. Thus, if we fix temperature and time, the dose (in natural logs) that will give a predicted 99.9968% mortality (probit 9) is:

$$\text{Ln dose} = [99.9968 + 17.7029 - 17.3150 (\text{Ln temp}) - 7.4915 (\text{Ln time})]/26.3601$$

Table 1. Insect mortalities and ethylene dibromide (EDB) residues in grapefruit when fumigated at 5 levels for each of 3 variables (dose, time, temperature).

Test no.	EDB dose[z]	Time (hr)	Temp (°F)	Insects[y] treated	Pupae recovered	Mortality (%)	Adults recovered	EDB[x] residue
1	0.25	1.01	80	1464	1722	0	971	0.23
2	0.25	2.13	56	1804	2551	0	718	0.13
3	0.25	2.13	65	4899	2202	55.04	130	0.31
4	0.25	2.13	74	1804	2110	0	901	0.26
5	0.25	3.24	74	1804	1752	0	997	0.37
6	1.4	1.01	56	2489	1077	56.73	132	0.43
7	1.4	1.01	56	3319	1224	63.12	26	0.74
8	1.4	1.01	74	3319	618	81.38	272	0.94
9	1.4	1.01	80	1464	262	82.11	119	0.90
10	1.4	2.13	65	1804	336	81.42	81	0.88
11	1.4	3.24	56	7091	428	93.96	140	1.82
12	1.4	3.24	74	3319	87	97.38	32	2.25
13	1.4	3.24	80	1464	4	99.73	0	1.96
14	3.9	0.25	65	4899	700	85.70	438	0.90
15	3.9	0.25	80	1464	98	93.31	37	1.38
16	3.9	1.01	50	3994	437	89.09	25	0.84
17	3.9	1.01	65	4899	62	98.73	26	2.11
18	3.9	2.13	50	3994	20	99.50	5	2.52
19	3.9	2.13	56	7091	7	99.90	1	3.39
20	3.9	2.13	65	4899	1	99.98	0	3.16
21	3.9	2.13	65	3337	7	99.97	0	2.87
22	3.9	2.13	65	8256	3	99.96	0	—
23	3.9	2.13	65	7224	3	99.96	1	—
24	3.9	2.13	65	8256	0	100	—	—
25	3.9	2.13	65	7224	3	99.96	0	—
26	3.9	2.13	74	1804	5	99.72	1	3.71
27	3.9	2.13	80	1464	0	100	—	3.83
28	3.9	4.0	65	4899	0	100	—	4.38
29	6.3	0.62	56	7091	131	98.15	21	3.42
30	6.3	1.01	50	3994	53	98.68	23	1.98
31	6.3	1.01	56	3319	23	99.31	8	2.42
32	6.3	1.01	74	3319	1	99.97	0	3.96
33	6.3	2.13	50	3994	4	99.90	0	2.33
34	6.3	2.13	80	1464	9	99.39	9	5.95
35	6.3	3.24	56	7091	0	100	—	9.03
36	6.3	3.24	74	3319	0	100	—	8.93
37	8	0.25	50	3994	386	90.35	18	1.07
38	8	0.25	56	7091	294	95.85	72	2.63
39	8	0.25	56	2913	59	97.97	12	1.13
40	8	0.25	80	1804	277	84.65	43	2.37
41	8	2.13	56	7091	0	100	—	11.34
42	8	2.13	65	4899	0	100	—	5.37
43	8	4.00	50	3994	0	100	—	5.42

[z]Ounces of ethylene dibromide/1000 ft^3.
[y]Number of insects treated is based on the average no. of insects/fruit recovered from the control times the number of fruit fumigated.
[x]Mean ppm EDB for 4 grapefruit.

The actual dose is obtained by taking antilogs. This has been evaluated for all possible combinations of temperatures between 50 and 80°F (in steps of 5°F) and of time between 2 and 3 hr (in steps of 0.25 hr) and presented in Table 2.

Table 2. Calculated ethylene dibromide (EDB) doses (oz EDB/1000 ft^3 airspace) needed to give 99.9968% insect mortality at some selected times and temperatures normally encountered in an actual grapefruit filled semi-trailer fumigation.[z]

	Time (hr)			
Temp (°F)	2.0	2.25	2.5	3.0
50	5.5	5.3	5.2	4.9
55	5.2	5.0	4.9	4.6
60	4.9	4.7	4.6	4.4
65	4.6	4.5	4.4	4.1
70	4.4	4.3	4.2	3.9
75	4.2	4.1	4.0	3.8
80	4.1	3.9	3.8	3.6

[z]Load factor is considered constant at 25%.

The multiple linear regression of EDB residue on the logarithms of time, temperature and dose gave a coefficient of determination of 0.6332. The equation is given below:

$$\text{residue} = 10.267 + 2.574\,(\text{Ln temp}) + 1.680\,(\text{Ln time}) + 1.790\,(\text{Ln dose})$$

Again, time and dose were more significant than temperature (significant at the 0.01%, 0.01%, and 13.0% levels respectively). The correlation coefficient between percent mortality and residue in the fruit was 0.49.

Linear regression of mortality on residue using the equation; % mortality = 66.81 + 590 (Ln residue), gave a correlation coefficient of only 0.24. Examination of Table 1 mortality and residue data indicates that when EDB residues were 2 ppm or greater (except test #40) insect mortalities exceeded 95%. In tests where the EDB residue was 3 ppm or more (except test #29 which was fumigated for only 0.62 hr because of a timing error) all tests exceeded 99% kill of insects. At EDB levels of 4 ppm and above, only test #34 had less than 100% mortality. Critical examination of procedures and raw data in this test did not reveal any obvious reason for the insect survival occurring with almost 6 ppm EDB present in the fruit. Four tests, numbers 27, 28, 42, and 43, all had lower EDB levels in the fruit than test #34, but had 100% insect mortalities.

King and von Windeguth (5) showed that a fumigation of grapefruit at the 8 oz dose rate for a 2-hr time period resulted in an EDB residue of ca. 12 ppm EDB in the fruit at the end of the 1-hr aeration period. This fruit, when held at 70-77°F, contained ca. 40 ppb EDB after 7 days and ca. 3 ppb after 21 days. Holding the fruit at a cooler temperature retarded the EDB loss. In fruit stored at 55°F, the EDB level fell from an initial level of ca. 17 ppm immediately following aeration to 9 ppb after 22 days of cold storage. EDB loss has been shown to be proportional to the amount present (5). The 4 oz/1000 ft^3 treatment reported in the present tests contained 4 to 6 ppm EDB immediately following the aeration period so the residues would have been <20 ppb at 7 days and <2 ppb at 21 days post fumigation if the 4 oz treatment lost EDB at the same rate as an 8 oz fumigation.

Two large chamber (9000 ft^3) semi-trailer fumigations were done in the spring of 1984 to simulate actual quarantine fumigations on full loads of fruit (1). A total of ca. 9700 insects contained in fruit were fumigated at the 4 oz/1000 ft^3 rate for 2.5 hr. No insects were recovered from the infested fruit. Further confirmatory tests will be done when fruit becomes available and a reasonably high insect infestation rate can be achieved.

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