TOXICITY OF ETHYLENE DIBROMIDE TO EGGS AND LARVAE OF THE CARIBBEAN FRUIT FLY, *ANASTREPHA SUSPENSA*[1]

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ABSTRACT

In laboratory studies ethylene dibromide (EDB) was found to be an effective fumigant against eggs and larvae of the Caribbean fruit fly, *Anastrepha suspensa* (Loew). Exposed eggs and larvae were killed by fumigations of less than 1 mg/l for 2 hr. Higher dosages of EDB were needed to kill eggs artificially protected by grapefruit rind and larvae artificially protected by half grapefruit. Adult emergence from puparia of exposed and protected larvae surviving treatment was reduced or prevented. These laboratory studies indicate that EDB could be used to fumigate fruit infested by Caribbean fruit fly eggs and larvae.

In Florida the Caribbean fruit fly, *Anastrepha suspensa* (Loew), is mainly a pest of dooryard, tropical, and subtropical fruits (Weems 1966). Occasionally infestations have been found in less preferred fruits such as grapefruit, *Citrus paradisi* Macf., mango, *Mangifera indica* L., and avocado, *Persea americana* Mill., (Swanson and Baranowski 1972). Therefore fumigation of commercial citrus and other fruit is required when they are being shipped to areas where the Caribbean fruit fly could survive (Burditt and von Windeguth 1975).

Small-scale laboratory fumigations, with 5-gal chambers, established the effectiveness of ethylene dibromide (EDB) against eggs and larvae of the Oriental fruit fly, *Dacus dorsalis* Hendel (Balock and Lindgren 1951). Additional research (Balock 1951) demonstrated the effectiveness of EDB as a fumigation treatment against eggs and larvae of the oriental fruit fly and other fruit flies infesting papayas, guavas, and tomatoes. As a result EDB was established as a commercial fumigant for quarantine use. EDB also was found effective as a treatment for fruits infested by eggs and larvae of the Mexican fruit fly, *Anastrepha ludens* (Lowe) (Shaw and Lopez 1954) and other *Anestrepha* spp. (Richardson 1952).

The objective of this study was to establish dosage-mortality values for EDB when used to fumigate Caribbean fruit fly eggs and larvae, exposed or artificially protected by grapefruit, in small-scale tests under laboratory conditions.

MATERIALS AND METHODS

Fumigations were conducted under ambient laboratory conditions (22-30°C) using 19.5 liter (5 gallon) glass bottles as test chambers (Fig. 1). The bottles were fitted with wood covers and a rubber washer for a tight

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3. This paper reports the results of research only. Mention of a pesticide or equipment in this paper does not constitute a recommendation for use by the U.S. Department of Agriculture nor does it imply registration under FIFRA as amended.

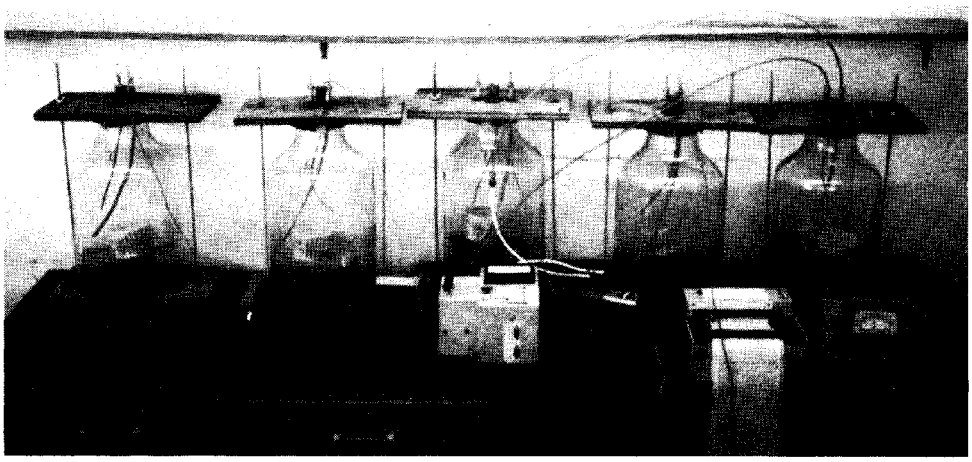

Fig. 1. 19.5 liter (5 gal) glass bottles as test chambers.

seal. Openings were provided in the cover for a gas inlet, gas outlet, and thermistor probe. Test specimens were supported in the bottles on a hardware cloth platform to allow optimum exposure to the gas. EDB concentrations were measured, to verify the applied dosage, with an infra-red spectrophotometer (Miran 1A Gas Analyzer, Wilks Scientific Corporation) and recorded on a strip chart recorder. EDB was injected into a 6.5 liter/min pump, where it evaporated and was delivered into the test chambers and Miran unit. Brass unions and nylon tubing were used in connections from the pump to other parts of the apparatus. Each test was replicated 5 times.

Exposed eggs were incubated (25°C) for approximately 24 hr prior to testing. Fifty eggs were placed on a small square of black construction paper which was placed on blotting paper and fumigated in open Petri dishes. Dosages of EDB tested were 0.0, 0.07, 0.12, 0.16, and 0.22 mg/l. After fumigation for 2 hr the eggs were left uncovered for approximately an hour to eliminate absorbed gas. The blotting paper was then moistened with 0.03% sodium benzoate solution, and the Petri dishes were covered. The eggs were examined 48 hr after exposure to determine mortality.

Eggs, artificially protected by grapefruit rind, were prepared and incubated in the same manner as for exposed egg tests. A 1-mm slice of grapefruit rind was placed over the square of black construction paper which contained the eggs, and was waxed onto the Petri dish. To prevent mold growth and dessication, 0.03% sodium benzoate solution was injected through the grapefruit rind. Dosages of EDB tested were 0.0, 0.25, 0.35, 0.45, and 0.55 mg/l. After fumigation for 2 hr, the grapefruit rind with the eggs under it was exposed to air for 24 hr. The grapefruit rind was then removed from the eggs, the Petri dish cover replaced, and the eggs were examined for hatch 48 hr after fumigation.

Twenty-five mature, 3rd instar larvae were put into 12-oz waxed cardboard ice cream containers, covered with saran screen, and fumigated with EDB for 2 hr at dosages of 0.0, 0.05, 0.20, 0.35, or 0.50 mg/l. Exposed larvae which did not pupate after 48 hr were considered dead. Larvae which pupated were left in the containers and subsequent emergence of adults was observed.

For tests in which larvae were artificially protected by half grapefruit, 25 mature, 3rd larval instars were put into a 5-ml glass beaker and covered with saran screen. Grapefruit were cut in half, a piece of the pulp was removed, and the beaker of larvae was inserted in the fruit. The periphery of the cut surface of the grapefruit was attached to a watch glass with masking tape. These larvae were fumigated with EDB for 2 hrs at dosages of 0.0, 1.0, 4.0, 7.0, or 10.0 mg/l. The half-grapefruit was left covering the larvae for 24 hr, at which time the larvae were removed and put in 12-oz, waxed, cardboard ice cream containers, covered with saran screen. The larvae were left in these ice cream containers for an additional 24 hr to permit pupation to ascertain the effect of EDB on adult emergence.

Data from these tests were analyzed by a probit program developed by Daum and Killcreas (1966) and modified at our laboratory by Rosa Lopez-Dellamary for a Wang 2200B.

Results and Discussion

Results of EDB fumigation of eggs and larvae of the Caribbean fruit fly, exposed and artificially protected by grapefruit rind, are given in Table 1. These results show that eggs are more susceptible to EDB than are larvae. A threefold increase in dosage of EDB was needed to attain the LD_{50} of eggs protected by grapefruit rind as compared to exposed eggs. A tenfold increase in EDB dosage was needed to attain the LD_{50} of larvae protected by half-grapefruit as compared to exposed larvae. Sorption and the mechanical protection of eggs by grapefruit rind and larvae by half-grapefruit were apparently part of the cause of increased dosages of EDB required.

The effect of the grapefruit on mortality of the eggs and larvae during fumigation can further be observed in the slopes of the dosage-mortality regression lines. The dosage-mortality regression line slope is steeper for exposed eggs and larvae than for the slope of those protected by grapefruit. The range of upper and lower confidence units indicate a more homogeneous response in exposed eggs and larvae to EDB as compared to the more variable, heterogeneous response of those protected by grapefruit. Owing to sorption and mechanical blockage of EDB molecules by grapefruit, there is a less direct exposure to EDB, causing a more varied response.

When exposed larvae were fumigated, adult flies emerged from 42.9% of the control puparia, compared to 39.6%, 7.4%, 0.0%, and 0.0% for those fumigated at 0.05, 0.20, 0.35 and 0.50 mg/l, respectively. When larvae were tested under half-grapefruit, adults emerged from 30.7% of the control puparia compared to 20.8%, 1.8%, 0.0%, and 0.0% of surviving puparia fumigated at 1.0, 4.0, 7.0, and 10.0 mg/l, respectively. Low emergence of flies from control puparia apparently was due to desiccation during the holding period.

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TABLE 1. EFFECTIVENESS OF EDB AS A FUMIGANT AGAINST CARIBBEAN FRUIT FLY EGGS AND LARVAE.

Stage fumigated	Dosage (mg/l)*						
		Limits**			Limits**		
	LD-50	Upper	Lower	LD-95	Upper	Lower	Slope
Exposed eggs	0.11	0.12	0.10	0.21	0.24	0.19	5.80
Eggs under grapefruit	0.31	0.37	0.22	0.69	1.77	0.53	4.75
Exposed larvae	0.29	0.32	0.26	0.71	0.91	0.60	4.30
Larvae under grapefruit	2.92	3.74	2.09	15.92	30.81	10.89	2.23

*Dosage required to prevent 50% and 95% hatch of eggs or pupation of larvae.

**95% confidence limits for lethal dosages.

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SHADOW SAMPLING: A FAST, PAINLESS METHOD FOR COLLECTING FALL ARMYWORM EGG MASSES—(*Note*).

After the release of the egg parasite *Telenomus remus* (Nixon) in S. Florida much time was spent attempting to recover it from field collected *Spodoptera frugiperda* (J. E. Smith) egg masses. *S. frugiperda* prefers young corn and generally oviposits on the underside of the leaf. Eggs are usually collected by removing the plant from the soil and inspecting it or by looking on the underside of the leaves with the plant in situ. While collecting egg masses, it was discovered they appear as dark spots showing through to the upper surface of the leaf when the plant is in the sampler's shadow. The technique of using one's shadow to find the egg masses is hereafter called the "shadow method". To use the shadow method the sampler simply walks so as to cast his shadow to one side and over the plants so the leaves can be inspected visually for eggs. This note compares the 3 methods mentioned above of sampling for *S. frugiperda* egg masses on corn.

Three collecting methods were compared for efficiency by sampling 8 plots of corn. The number of egg masses found and the time required to sample each plot were recorded. Each plot was 50 ft long with plants approximately 6 in. apart in the row. The plants were stage 5 (J. J. Hamway, 1966, Iowa St. U. Special Rep. no. 48) when the methods were compared. First the plots were sampled by the shadow method, then the undersides of all the leaves were inspected, and finally all plants in the plots were pulled and examined.