Methyl Bromide Fumigation Quarantine Treatment for Carambolas Infested with Caribbean Fruit Fly (Diptera: Tephritidae)

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ABSTRACT A methyl bromide fumigation quarantine treatment was developed for 'Arkin' carambolas, *Averrhoa carambola* L., infested with the Caribbean fruit fly, *Anastrepha suspensa* (Loew). Although 1 larva of an estimated 104,303 in 6,796 carambolas survived fumigation at 40 g/m^3 for 2 h (temperature 23 ± 1°C) it died as an apparently normal pupa. No immediate detrimental effects were observed on sound carambolas after fumigation; however, shelf life at room temperatures was reduced by 24–30%. Methyl bromide residues in carambolas fumigated with 40 g/m^3 for 2 h and stored at 23 ± 1°C were not detected (<10 ppb) after 48 h. After 48 h of storage at 4.5 ± 0.5°C, fumigated carambolas had residues of 0.83 ± 0.09 ppm. Methyl bromide fumigation of carambolas with 40 g/m^3 for 2 h at 23 ± 1°C could be a viable quarantine treatment of carambolas infested with the Caribbean fruit fly if the fruit fly mortality and carambola shelf life observed in this study were not objectionable.

KEY WORDS Insecta, *Anastrepha suspensa, Averrhoa carambola,* quarantine security

CARAMBOLA, *Averrhoa carambola* L., is a tropical fruit in increasing demand. As a host of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), carambolas grown in Florida are subject to quarantine regulations when shipped to areas where the Caribbean fruit fly does not exist and might become established. Carambolas exposed to 1.1°C for 15 d to kill Caribbean fruit flies have been shipped to California (Gould & Sharp 1990), although damage has occurred to some carambolas because of this quarantine treatment (C. A. Campbell, personal communication). Quarantine treatments with hot water immersion, vapor heat, and hot air have also been developed (Hallman 1990b, Hallman & Sharp 1990, Sharp & Hallman 1992). Hallman (1990a) found that some cultivars of carambolas would not tolerate hot water immersion. These cultivars also may not tolerate vapor heat or hot air.

Methyl bromide is used widely as a quarantine fumigant (Animal and Plant Health Inspection Service 1980). To our knowledge, no reports on methyl bromide fumigation of carambolas have been published. The objectives of our studies were to develop a methyl bromide fumigation quarantine treatment for carambolas infested with Caribbean fruit fly and to examine the effects of methyl bromide fumigation on the quality of carambolas.

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Materials and Methods

Fumigation Chamber. Fumigation chambers with a capacity of 0.8 m^3 described by Benschoter (1979) were used in this research. The chambers were constructed of sealed plywood (6.5 mm thick) over steel frames. Ducting for recirculating the air was made of PVC pipe (5 cm diameter). Liquid methyl bromide under pressure in a cylinder was measured volumetrically and then injected into the fumigation chamber, where it expanded into a gas. A blower circulated the air from top to bottom at the rate of approximately two chamber volumes per minute. Methyl bromide concentrations in the chambers were determined with a Fumiscope (Model E-200; R. K. Hassler Company, Altadena, Calif.).

Fumigations were performed at atmospheric pressure and 23 ± 1°C. Carambolas were kept at 23 ± 1°C and were dry on the surface when placed in the chambers.

Determination of Concentration to Kill Fruit Fly. Two experiments were done to estimate the concentration of methyl bromide necessary to kill Caribbean fruit fly immatures in carambolas. In one experiment, carambolas infested with second and third instars were fumigated. In the other experiment, carambolas infested primarily with eggs 24 h or older were fumigated.

'Arkin' carambolas (weight range, 51.2–207.7 g; mean 112.7 g) were provided by J. R. Brooks & Son, Homestead, Fla. They were infested with Caribbean fruit flies by being placed for 2–3 d in an outdoor screened cage (3.5 by 3.5 by 2.5 m) with ≈200,000 flies. The flies were reared on an agar-based diet (85% of the flies) and a corn cob-based diet (15% of the flies) (Burditt et al. 1975). Temperature within the cage ranged between 21 and 32°C.

In the test in which mostly eggs were fumigated, carambolas were removed from the infestation cage, held in a room at 23 ± 1°C for 1 d, randomly separated into seven groups of equal numbers, and fumigated with methyl bromide at the rates of 8, 12, 16, 20, 24, or 28 g/m^3 at 23 ± 1°C for 2 h. One group of carambolas was not fumigated and was used to estimate the number of Caribbean fruit fly immatures present. The load factor was ≈15% of the chamber volume. The test was conducted twice with 34 and 51 fruit per treatment.

In the test where carambolas containing older instars were fumigated, the carambolas were held at 23 ± 1°C for 5 d after they were removed from the infestation cage and before they were fumigated. The rest of the test was treated in the same manner as the previous test and was done twice with 30 fruit per treatment.

In both tests, the fumigated and nonfumigated carambolas were placed in cages (Hallman & Sharp 1990) to collect surviving larvae. To estimate the number of larvae of the Caribbean fruit fly killed at each dose, the number of survivors at each dose was subtracted from the number of larvae recovered from the nonfumigated carambolas. Larvae were placed in plastic containers (0.5 liter) with 0.1 ml of moist vermiculite and held for pupation and adult emergence.

Data were subjected to probit analysis (SAS Institute 1988, 149–170). Probit 9 (99.9968%) mortality and 95% fiducial limits (FL) were estimated with methods described by Finney (1971).

Confirmation of Concentration to Kill Fruit Fly. After estimates of the concentration necessary to achieve probit 9 mortality were obtained, >100,000 Caribbean fruit fly immatures infesting carambolas were fumigated with 40 g/m^3 of methyl bromide at 23 ± 1°C for 2 h. The chambers were loaded to 50–75% of capacity to more closely simulate commercial conditions. Carambolas were placed in the infestation cage for 2–5 d, then held at 23 ± 1°C for 1–5 d so that all immature stages were subjected to fumigation. Ten percent of the infested carambolas selected at random were not fumigated but were placed in cages to estimate the infestation level of Caribbean fruit fly immatures. The fumigated carambolas were placed in cages to recover surviving larvae.

Quality of Methyl Bromide-Fumigated Carambolas. 'Arkin' carambolas were randomly separated into three groups of 40 carambolas per group. Two of the groups were fumigated with methyl bromide at 40 g/m^3 and 23 ± 1°C for 2 h, one group inside the shipping boxes and one group in plastic trays. The third group was not fumigated. All groups were placed in storage at 4.5 ± 0.5°C for 6 d and then maintained at 23 ± 1°C until they ripened. Periodic qualitative observations were made to evaluate fruit appearance and the incidence of decay after harvest. Fruits were cut open and qualitative observations on internal appearance and taste were made. This test was replicated four times. Fruits without fungal growth detectable to the unaided eye were considered to be marketable. The number of days to the time when 80% and 50% of the carambolas was still marketable were analyzed with analysis of variance (ANOVA) (SAS Institute 1985).

Methyl Bromide Residue Analysis. Methyl bromide residues in fumigated carambolas were determined by the headspace method (King et al. 1981). Four corrugated cardboard shipping cartons (34 by 32 by 14 cm), each containing 20 carambolas (mean weight, 145 g; range, 98–203 g), were fumigated with 40 g/m^3 at a temperature of 23 ± 1°C for 2 h. Each carton end had two oblong holes (1 by 4.5 cm), and the top and bottom had four oblong holes of the same size. The inside bottom and top of the carton was covered by perforated foam plastic (4 mm thick). The carambolas were wrapped in thin paper and placed stem end down in 20 compartments formed by interlocking pieces of cardboard. Fruits from two of the cartons were removed from the cartons and placed on plastic mesh trays before fumigation to determine the effect of the carton on absorption of the methyl bromide by the carambolas. One of the lots of carambolas fumigated inside the carton and one fumigated in the plastic trays were stored at 4.5 ± 0.5°C; others were stored at 23 ± 1°C. The test was replicated three times.

Methyl bromide residues were determined at 1, 3, 5, 24, 48, and 72 h after fumigation for the carambolas stored at 23 ± 1°C and 24, 48, and 72 h after fumigation for the carambolas stored at 4.5 ± 0.5°C. A 100-g sample of carambola and 100 ml of water were blended in a 500-ml glass container sealed with a Teflon-lined lid fitted with a septum. After 20 min (to allow mists to settle), a 5-ml sample of the headspace gases was removed and injected into a 0.5-ml loop of a gas sampling valve in a gas chromatograph (Model 5730; Hewlett-Packard Company, Avondale, Pa.). A glass column (4 mm inside diameter, 1 m long) packed with 100–120 mesh Porapak Q (Waters Associates, Milford, Mass.) was used with a linear Ni^{63} electron capture detector. Standards were prepared with identical samples of unfumigated fruit with various levels of methyl bromide in the blender jar before blending to compensate for the partitioning of methyl bromide between the aqueous and liquid phases. The system allowed for detection of concentrations as small as 10 ppb.

Table 1. Analysis of methyl bromide fumigation (at 23 ± 1°C for 2 h) of 'Arkin' carambolas infested with Caribbean fruit fly immatures

Test	Estimated no. immatures/dose	Estimated no. immatures killed at g/m³					
		8	12	16	20	24	28
			Eggs[a]				
1	427	—	135	271	375	421	425
2	3,377	1,991	3,309	3,334	3,368	3,377	3,377
			Larvae[b]				
1	1,941	1,799	1,879	1,912	1,930	1,936	1,940
2	2,382	2,339	2,214	2,371	2,379	2,379	—

[a] Probit 9 (linear time) = 29.6 g/m^3; 95% FL = 24.3–41.7 g/m^3; slope ± SE = 0.17 ± 0.024. Probit 9 (log base 10) = 44.2 g/m^3; 95% FL = 33.9–66.6 g/m^3; slope ± SE = 5.03 ± 0.41.

[b] Probit 9 (linear time) = 38.6 g/m^3; 95% FL = 26.7–134.1 g/m^3; slope ± SE = 0.080 ± 0.022. Probit 9 (log base 10) = 91.4 g/m^3; 95% FL = 32.3>1,000 g/m^3; slope ± SE 2.32 ± 0.80.

Results

Determination of Concentration to Kill Fruit Fly. An estimated 3,804 eggs and 4,323 larvae per concentration were treated in the tests of egg mortality and larval mortality, respectively. These numbers exceeded the 1,000–1,500 insects per dose recommended by Chew & Ouye (1985). Results of probit analyses are presented in Table 1. Upper 95% FL varied from 41.7 g/m^3 (egg kill, linear time) to >1,000 g/m^3 (larval kill, log base 10 time). The wide range in probit 9 estimates and upper FLs made it difficult to select a dose for fumigation of carambolas infested with Caribbean fruit fly, in large part because probit 9 was an extreme end of the tolerance distribution. Much of this variation also resulted from two sources of uncontrollable error: (1) the population level was unknown and and assumed to not vary between treatments in the same replicate when, in reality, it varies by as much as twice or half the estimated values (G. J. H., unpublished data); and (2) natural mortality is assumed to be zero. These errors are inherent in this type of research. When data from egg and larval kills were analyzed together, the probit 9 estimate for linear time was 32.1 g/m^3 (95% FL = 29.6–35.3 g/m^3; slope ± SE = 0.13 ± 0.0062). The probit 9 estimate for log base 10 time was 53.2 g/m^3 (95% FL = 43.6–69.2 g/m^3; slope ± SE = 3.88 ± 0.20).

These results suggested that the probit 9 estimate for linear time (up to 35.3 g/m^3 for the upper 95% FL) was too low because larvae were recovered at the dose of 28 g/m^3 (Table 1). The probit 9 for log base 10 time (up to 69.2 g/m^3) was probably too high based on doses of methyl bromide needed to kill fruit flies in grapefruit (Benschoter 1979, Williamson et al. 1986), stonefruits (Armstrong & Couey 1983), and papayas and pears (Spitler & Couey 1983). Therefore, we chose a dose of 40 g/m^3 of methyl bromide for the confirmatory test necessary to achieve probit 9 security (Couey & Chew 1986). Our decision was based on the fact that this dose was approximately midway between the 95% FL ranges for linear time and log base 10 time when all of the data were analyzed together, and that the dose appears consistent with those used in studies (previously cited) involving methyl bromide treatments of fruits infested with fruit flies.

Confirmation of Concentration to Kill Fruit Fly. A total of 6,796 carambolas (weight range, 51–208 g; mean weight, 113 g) infested with the Caribbean fruit fly were fumigated with 40 g/m^3 of methyl bromide at 23 ± 1°C for 2 h. One of an estimated total of 104,303 eggs and larvae of the Caribbean fruit fly in these carambolas survived the fumigation. The survivor pupated but died before the adult emerged. Emergence of adults from pupae from untreated carambolas was 49%.

Quality of Methyl Bromide-Fumigated Carambolas. No qualitative differences in color, taste, or appearance were observed between treated and untreated carambolas 1–2 d after treatment. However, fumigated fruit had an increased rate of ripening and decomposition (Table 2).

Methyl Bromide Residue Analysis. The results of methyl bromide residue analyses are given in Table 3. No residues were detected in carambolas stored at 23 ± 1°C after 48 h, whereas carambolas stored at 4.5 ± 0.5°C had a residue of 0.04 ppm after 72 h. Residues 1 h after fumigation were virtually identical for carambolas fumigated in shipping cartons (17.8 ± 0.9 ppm) versus those fumigated in open plastic trays (17.6 ± 2.5 ppm). Linear regression analysis of the logarithm of residue concentration over time gave correla-

Table 2. Days to 80 and 50% of carambolas still marketable after fumigation with 40 g/m³ methyl bromide at 23 ± 1°C for 2 h

Treatment	Mean no. d to % marketable fruit	
	80%	50%
Control	5.0z	6.7z
Fumigation out of carton	3.5y	5.1y
Fumigation in carton	3.4y	5.1y

Means in same column followed by same letter are not significantly different $\alpha = 0.05$; Ryan-Einot-Gabriel-Welsch multiple *F* test (SAS Institute 1985).

Table 3. Methyl bromide residues in 'Arkin' carambolas after fumigation with 40 g/m³ at 23 ± 1°C for 2 h and subsequent storage at two temperatures

H after fumigation	Residue (ppm ± STD) at storage temperatures	
	23 ± 1°C	4.5 ± 0.5°C
1	18.1 ± 1.7	—
3	11.4 ± 0.9	—
5	8.6 ± 0.3	—
24	0.17 ± 0.04	3.06 ± 0.23
48	Not detected	0.83 ± 0.09
72	Not detected	0.04 ± 0.01

tion coefficients of 0.9994 (slope ± SE = −0.203 ± 0.0034) and 0.9498 (slope ± SE = -0.090 ± 0.021) for 22–24°C and 4–5°C storage, respectively. The calculated half life of methyl bromide was 3.4 h at 22–24°C and 7.7 h at 4–5°C.

Discussion

One larva of an estimated 104,303 Caribbean fruit flies survived fumigation with 40 g/m³ methyl bromide at 22–24°C for 2 h, although the resulting pupa died. This would meet probit 9 security at the 95% confidence level (no survivors of 93,613) (Couey & Chew 1986) if the criterion for survival was based on the number of adults that survived fumigation. Landolt et al. (1984) and Chew & Ouye (1985) argued that probit 9 security was extreme when required in a commodity that was rarely infested with fruit flies in commercial orchards. Although carambolas were readily infested with Caribbean fruit flies during this research, the fruit have rarely been found to be infested in commercial orchards (W. P. Gould, unpublished data).

The fact that shelf life of carambolas at room temperature was reduced by methyl bromide fumigation restricts, but does not eliminate, the usefulness of this quarantine treatment. Fumigation of carambolas in the shipping cartons has no apparent effect on the amount of methyl bromide absorbed; carambolas could thus be fumigated after packing.

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