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Efficacy of methyl bromide and cold storage as disinfestation treatments for guavas infested with Caribbean fruit fly

Guy J. Hallman
USDA/ARS Subtropical Agricultural Research Center, 2301 S. International Blvd, Weslaco, TX 78596, USA

Abstract *Methyl bromide (MB) fumigation and cold storage were investigated as treatments to disinfest guavas of Caribbean fruit fly,* Anastrepha suspensa *(Loew). Cold storage at 1 or 3°C for 16 days resulted in excessive chilling injury damage and lack of ripening compared with the control at 7°C. Guavas tolerated MB fumigations up to 20 g/m³ for 2 h at 24°C. However, that dose did not provide quarantine security, defined as 99.9968% mortality with 95% confidence and estimated to be attained at 28–33 g/m³. Although quarantine security was not achieved with MB fumigation alone without unacceptable damage to the guavas, perhaps it could be achieved with a combination treatment involving no more than 20 g/m³ of MB coupled with some other treatment, such as heat or irradiation.*

Keywords: guava, *Anastrepha suspensa*, Caribbean fruit fly, quarantine treatment, postharvest treatment.

Introduction

Fruits that are hosts of tephritid fruit flies must be subjected to treatments that will kill virtually 100% of any fruit fly eggs and larvae that might be in the fruits before they can be shipped to any place that has a quarantine against the flies. The Caribbean fruit fly, *Anastrepha suspensa* (Loew), occurs in several Caribbean countries (Weems 1966), and guava, *Psidium guajava* L., is a preferred host with commercial export value (Swanson and Baranowski 1992). To my knowledge, no quarantine treatment is currently used for guavas for any pest in any location. Research has been performed on heated air fruit fly disinfestation (Seín 1935; Gould 1994b). Guavas might be disinfested with irradiation: Ahmad *et al.* (1972) found that 0.3 kGy was optimum for prolonging shelf life of guavas, and that dose should prevent adult emergence of any fruit flies infesting guavas (Hallman 1998). Although a hot water immersion treatment for guavas has been developed (Gould and Sharp 1992) and approved by California, it cannot be used if more than 1% of the guavas are infested. Guavas usually have an infestation rate greater than 1%. A pre-treatment infestation rate limit of 1% would not be demanded for a methyl bromide (MB) fumigation quarantine treatment. Although MB is considered a significant stratospheric ozone depleter, the Montreal Protocol has exempted its use in postharvest disinfestation.

This research evaluated the effects of postharvest cold storage and MB fumigation on guava quality and the potential of these two procedures as quarantine treatments for guavas infested with Caribbean fruit fly.

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Materials and methods

Experiments were done to estimate the dose necessary to achieve quarantine security (effective dose that provides 99.9968% mortality or $ED_{99.9968}$) with MB fumigation and to measure the response of guava fruits to this fumigation and cold storage. This level of security is commonly demanded by the US Animal and Plant Health Inspection Service (Shannon 1994). Guavas cv. Ruby were obtained from Sardiña Farms and J.R. Brooks & Son, Inc., in Homestead, Florida.

Forty guavas each were fumigated with 20, 25, 30, 36 or 44 g/m^3 MB for 2 h at 24±0.4°C, which are standard conditions used for fumigating Caribbean fruit fly-infested citrus in Florida (Hallman and Chalot 1993). Another lot of 40 guavas was not fumigated (control). After fumigation, all of the guavas were held at 24±0.4°C and observed for quality. The number in each lot that showed 'bronzing', sunken, dark spots, anthracnose and ripening were counted periodically. Bronzing is a reddish-brown colouring of the peel. The degree of bronzing, spotting and anthracnose per fruit can vary between 0 and 100%, and minor damage is inconsequential to marketability. Therefore, a threshold was set at 10% of the surface covered with the particular damage before the fruit was counted as damaged. The test was repeated four times, and the data were subjected to analysis of variance as a randomized complete block design (SAS Institute, Cary, NC, USA).

Forty guavas each were stored at 1, 3 and 7±0.4°C for 16 days. Storage at 7°C is the control temperature at which guavas are stored and does not provide sufficient fruit fly mortality to serve as a disinfestation treatment (Benschoter and Witherell 1984). Vazquez-Ochoa and Colinas-Leon (1990) found that 7°C preserved guava quality better than 3.5 or 11°C. After 16 days, all of the guavas were held at 24±0.4°C until ripe and observed for quality. The number in each lot that showed chilling injury, anthracnose and ripening were counted periodically. Chilling injury to guavas results in sunken black spots (pitting) and imparts a dull, reddish-yellow colour to ripening fruits. Again, a threshold of 10% of the surface area was set for chilling injury or anthracnose before the fruit was counted as damaged. The test was repeated four times, and data were subjected to analysis of variance as a randomized complete block design.

Guavas were placed for 24 h in a walk-in infestation cage stocked with approximately 200 000 Caribbean fruit fly adults from a colony reared on semi-artificial diet (Hallman and King 1992). The guavas were held for 7 days at 24±0.4°C to obtain third instars, which were harder to kill with methyl bromide than eggs (Hallman and King 1992). The guavas were randomly separated into six groups of equal numbers, five of which were fumigated with 12, 20, 28, 36 and 44 g/m^3 MB at 24°C for 2 h. The sixth group was not fumigated and was used to estimate the number of Caribbean fruit fly larvae in the fumigated groups. All six groups of guavas were placed in plastic trays in metal towers fitted with a large funnel at the bottom. A plastic bin with about 0.5 l of sand was placed under the funnel; larvae emerged from the guavas and dropped into the bin to pupariate. Larvae and puparia were sifted from the sand, counted and kept to observe adult emergence. Mortality at each fumigation dose was estimated by subtracting the number of insects emerged per dose from the number emerged from the group of guavas that was not fumigated. The test was repeated three times with 111, 130 and 124 guavas per treatment. Data were subjected to probit analysis (SAS).

Results

Considerably more bronzing and spotting were observed on guavas fumigated with 25 or 30 g/m³ MB than on non-fumigated guavas or those fumigated with 20 g/m³ (Table 1). There were no significant differences in incidence of anthracnose or number of days required for guavas to ripen. Fewer guavas fumigated with 25 or 30 g/m³ MB ripened compared with guavas from the other treatments. Cold storage at 1 or 3°C resulted in greater chilling injury and fewer guavas ripening than at the control temperature of 7°C (Table 2). There was no significant difference in anthracnose incidence. Guavas stored at 7°C ripened quicker than those stored at lower temperatures.

Numbers of Caribbean fruit fly larvae emerging from the untreated guavas for the three replicates were 1249, 884 and 2145, sequentially. The estimated % mortality for each fumigation dose in all three replicates is in Table 3. The data fit the probit model whether dose or the $\log_{10}$ of dose were used in the analysis (prob. > χ^2 = 0.99 for both). Upper 95% fiducial limits for the estimated effective dose to kill 99.9968% of Caribbean fruit fly larvae in guavas were 28.0 and 32.7 for dose and $\log_{10}$ dose, respectively.

Table 1. Quality of guavas fumigated with methyl bromide and stored at 24°C until ripe*

Dose (g/m³)	Bronzing (%)	Spotting (%)	Anthracnose (%)	Ripened (%)	Days until ripe
0	0±0	7±1.2	6.9±0.6	90±3.4	7.3±1.0
20	6±1.6	14±4.6	6.3±0.7	93±1.4	7.5 ±1.3
25	68±7.8	77±6.3	4.4±0.6	84±2.4	6.8±0.5
30	86±2.4	91±3.9	5.0±1.8	63±3.2	7.5±1.0

*Values are means ± SEM of four replicates.

Table 2. Quality of guavas stored at three temperatures for 16 days and then stored at 24°C until ripe*

Temperature (°C)	Chilling injury (%)	Anthracnose (%)	Ripened (%)	Days until ripe
7	11±1.6	14±2.6	89±1.6	4.8±0.3
3	58±5.2	16±4.1	65±6.5	6.5±0.6
1	93±3.2	14±2.4	28±4.3	7.5±0.3

*Values are means ± SEM of four replicates.

Table 3. Estimated percentage mortality of Caribbean fruit fly infesting guavas fumigated with methyl bromide (MB)*

Replicate	% mortality at dose (g MB/m³)				
	12	20	28	36	44
1	97.4	100	100	–	100
2	90.5	99.8	100	100	100
3	97.2	99.9	100	100	100

*$ED_{99.9968}$(dose) = 24.4g/m³; upper 95% fiducial limit = 28.0g/m³; slope ± = 0.18 ± 0.021.
$ED_{99.9968}$($\log_{10}$dose) = 26.3g/m³; upper 95% fiducial limit = 32.7g/m³; slope ± = 6.64 ± 0.75.

Discussion

Guavas did not tolerate cold storage at ≤3°C for 16 days. It is doubtful that significantly shorter treatments or higher temperatures would provide quarantine security against the Caribbean fruit fly (Gould 1994a). An MB dose of 20 g/m^3 might be acceptable with regard to the market quality of guava, but it will not provide quarantine security at the $ED_{99.9968}$ level. The $ED_{99.9968}$ necessary to provide quarantine security of Caribbean fruit fly in guavas in 2 h at 24°C seems to be about 30 g/m^3. According to our study, guavas suffered significant damage at that dose; 37% did not ripen properly at 30 g/m^3. Perhaps a dose of 20 g/m^3 could be combined with another quarantine treatment to achieve quarantine security of guavas. Methyl bromide has been combined with cold storage or controlled atmosphere quarantine treatments of fruits such as citrus and grapes (Mangan and Sharp 1994).

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