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QUARANTINE TREATMENT RESEARCH AGAINST THE CARIBBEAN FRUIT FLY IN CITRUS FRUITS

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ABSTRACT

This review summarizes recent research findings with approved as well as potential quarantine treatments to replace ethylene dibromide (EDB) fumigation in the eradication of the Caribbean fruit fly *(Anastrepha suspensa* (Loew). Several million boxes of grapefruit *(Citrus paradisi* Macf.*)* are shipped annually from Florida to Japan; EDB fumigation and the cold treatment are the only approved methods to disinfest fruit of the Caribbean fruit fly. Limitations were found in the commercial use of the cold treatment, because of the presence of cold injury, in cold-intolerant cultivars such as grapefruit. In domestic shipments methyl bromide (MB) has been used for citrus fruits. To avoid phytotoxicity, citrus fruits fumigated with MB must be stored and handled at temperatures higher than those usually recommended. Gamma irradiation showed potential; presently it is not an approved treatment for fruits and vegetables. Phosphine (PH_3) fumigation, which is not approved for citrus fruits, is phytotoxic to grapefruit at conditions required for Caribbean fruit fly mortality.

RESUMEN

Se reportan los resultados de investigaciones recientes que examinaron diferentes tratamientos aprobados y experimentales par remplacer el uso de dibromuro de etileno (EDB) en la eradicación de la mosca del caribe *(Anastrepha suspensa* Loew.*)* Cada año se manda varios millones de cajas de toronja *(Citrus paradisi* Macf.*)* de Florida a Japón; fumigación con EDB y tratamiento con temperaturas bajas son los únicos metodos aprobados para desinfestar la fruta de la mosca del caribe. El uso comercial de temperaturas bajas es limitado debido al daño causado por el frío en especies susceptibles tales como la toronja. En cargamentos domesticos, se ha usado bromuro de metileno (MB) en cítricos. Para evitar fitotoxicidad se ha tenido que almacenar y manejar las frutas fumigadas con MB a una temperatura mas elevada de lo que se recomienda normalmente. La irradiación con rayos gama es un tratamiento potencial, pero todavía no es un tratamiento aprobado para frutas y verduras. Fumigación con Fosfina (PH_3) no es un tratamiento aprobados para cítricos, y es fitotóxico a la toronja en condiciones requeridos para matar la mosca del caribe.

Because of its importance in both domestic and export markets, Florida grapefruit, *Citrus paradisi* Macf., has been the focus of much of the research effort on quarantine treatment methods. Since 1975 Florida citrus fruits have been fumigated with ethylene dibromide (EDB) to eliminate possible infestations of Caribbean fruit fly, *Anastrepha suspensa* (Loew), in fruit shipped to Japan. EDB was used as a domestic quarantine treatment for the control of fruit flies in fruits and vegetables until its registration was cancelled for most uses effective September 1, 1984. Subsequent to cancellation, the U.S. Environmental Protection Agency (EPA) restricted the use of EDB to citrus destined for markets outside the U.S. beginning October 1 until January 31 of the following year. In 1985 such use was extended to May 31, 1985.

Although the cold treatment is approved for citrus fruits, its use for grapefruit shipments to Japan during the 1983–84 season proved to be disastrous. Exporters have reported that losses due to excessive cold injury ran into millions of dollars. Cold-tolerant cultivars such as 'Valencia' orange are readily adaptable to the treatment. No grapefruit has been shipped to Japan using the cold treatment since the problem was encountered. Japanese importers have requested U.S. exporters to fumigate grapefruit with EDB and not use the cold treatment.

While there is no Federal quarantine on the Caribbean fruit fly, the other citrus-producing states require treatment of Florida citrus fruits to rid the fruit of the fly as a condition of entry into their states. Methyl bromide (MB) fumigation has been used with limited success. Phytotoxicity problems have been encountered with oranges and specialty citrus fruits, but grapefruit shipments have been successful as long as temperatures above usual storage temperatures were used. Research data have been provided to the Government of Japan on the effectiveness of MB for the Caribbean fruit fly; additional approval has been received provided commercial tests are conducted. The future use of EDB as a fumigant continues to be uncertain, although low-dose fumigation was found to be effective for the control of Caribbean fruit fly in grapefruit; such doses also resulted in low residue levels (39). It is imperative that modifications of present treatments or new alternate treatments be found to replace EDB.

Phosphine (PH_3)

PH_3 fumigation is used commercially to fumigate against weevils in grain. In Hawaii, PH_3, effectively eliminated the Oriental and Mediterranean fruit flies from fruits other than citrus, and time influenced efficacy more than concentration (35). Studies (37, 38) showed that PH_3 generated from magnesium phosphide FUMI-CELS® could possibly be used as a quarantine fumigant for grapefruit. The tests showed that PH_3 gave 99 to 100% mortality to Caribbean fruit fly after 24-hr exposure to a concentration of 300 to 600 ppm for the first 6 to 8 hr, 73 to 200 ppm concentration during the remainder of the 24-hr period (38). Other tests indicated that Caribbean fruit flies infesting 'Marsh' grapefruit were controlled when fruit were fumigated at 13°C for 96 hr or fruit were fumigated at ambient temperature for 48 hr (37).

In further tests (26) dosages of PH_3 required to eradicate the Caribbean fruit fly caused phytotoxic effects to Florida grapefruit. These efforts were manifested as various forms of rind injury, of which the most serious was rind breakdown (Table 1). Rind breakdown includes pitting and aging; pitting is commonly manifested as dark, sunken, surface

Table 1. Rind breakdown and subsequent decay of 'Marsh' grapefruit fumigated with phosphine[z] (26).

Treatment	Phosphine[y] concentration ppm	Rind breakdown after storage (10°C) 28 days %	Decay after holding (21°C) 7 days %
Refrigeration (ambient 24 hr + 4 days at 10°C)	734	20.0a[x]	14.5a[x]
Ambient (ambient for 3 days)	325	16.8a	8.6ab
Control	0	2.5b	4.9b

[z] Each numerical value represents 12 boxes of fruit (480 fruit), 6 boxes from each of 2 grove sources with half of each fumigated at USDA Orlando.
[y] Average concentration of phosphine generated after 24 hr of fumigation.
[x] Mean separation within columns by Duncan's multiple range test, 5% level.

lesions, whereas aging is found at the stem end around the button in the form of wilt, shrivel, and collapse. Fruit fumigated under ambient conditions had less rind breakdown than similar fruit fumigated under refrigerated conditions. Rind breakdown did not appear until the fruit had been in refrigerated storage at 10°C. During holding for 7 days at 21° significantly more decay was found in fruit fumigated under refrigerated conditions; the extent of decay appeared to be proportional to the amount of rind breakdown that occurred during storage (26).

The phytotoxic effects appear to preclude any possibilities of PH_3 as a practical, commercial fumigant for Florida grapefruit. Also, the length of the PH_3 fumigation period is a major impediment to its use (48 to 72 hr minimum exposure above 20°C).

Methyl bromide (MB)

California grapefruit were reported to be uninjured by MB applied at rates of 2.5 lb/1000 ft^3 at 27°C and 3 lb/1000 ft^3 at 21°(1). Grapefruit were marked and discolored by high rates, and 'Washington' navel oranges (*Citrus sinensis* (L.) Osbeck) were spotted or discolored at all rates of MB application. Another report from California indicated that a 2-hr treatment with 2 lb of MB was an injurious concentration to fumigate citrus fruits for insect infestations, although lemons grown in the interior or California that were fumigated at this rate were uninjured (29).

With the Caribbean fruit fly in Florida MB fumigation rates of 40 and 56 mg/liter provided quarantine security for 20 and 80% chamber loading, respectively (3). However, using these rates MB was found to be too phytotoxic to 'March', 'Ruby Red' and 'Thompson Pink' grapefruit; 'Hamlin', 'Pineapple' and 'Valencia' oranges, and 'Temple' (*C. reticulata* X *C. sinensis* (?)) (17). Fumigation of grapefruit with MB resulted in peel injury during storage, especially at lowest temperatures (Table 2). The injury was manifested as scald and/or, in a few instances, discolored pitting of the peel. The scaldlike injury became water-soaked in severe instances. Occasionally, an entire lot of fruit would escape MB injury during 4 weeks of storage (simulated transit) but develop excessive decay after holding at 21°C for 7 days. This was especially the case for 'Hamlin', 'Pineapple' and 'Valencia' oranges and 'Temple'. With oranges and 'Temple' the development of symptoms was usually delayed and a general softening of the fruit was detected before symptoms of injury became visible; excessive decay followed.

Recent studies have shown that the combination of MB fumigation and cold storage treatment was effective for controlling infestations of Caribbean fruit fly in grapefruit (4). Commercial scale tests were conducted with MB as a fumigant for Caribbean fruit fly with uniform distribution of MB within a chamber fumigated with 40 g/m^3 for 2 hr in 266 m^3 (6). Residues of MB in fruit were calculated to reach 10 ppb and 1 ppb after 10 and 14 days, respectively, when fruit were stored at 16°C following fumigation.

Gamma irradiation

Gamma irradiation was proposed as a possible quarantine treatment for fruit infested with fruit flies in 1956 (2). The greatest concern was centered on possible injurious effects of irradiation on the fruit itself. Previous work with grapefruit and other fruit indicated that radiation when followed by cold storage or cold temperature shipment may cause injury to the peel (7, 11, 12, 13, 22). Preliminary investigations showed that 25 to 60 kilorad (krad) increased pitting, scald, aging and decay of Florida grapefruit (8). Additional research confirmed that dosages of 60 and 90 krad caused injury, although 15 and 30 krad dosages were acceptable (24, 25). Scald and, especially, rind breakdown of the peel were the types of injury which developed during the storage period. The magnitude of injury at various months of the season are shown according to dosages (Table 3). Biochemical tests showed an improvement of flavour in grapefruit sections, especially at lower dosages (30). No differences were noted in vitamin C content, sugar or acid levels in juice, nor in essential peel oil composition of volatile constituents from irradiated fruit when compared with those from untreated fruit.

A study of effects of irradiation on the mortality of the Caribbean fruit fly showed that none survived grapefruit irradiated at 60 and 90 krad, whereas one

Table 2. Methyl bromide fumigation of Florida grapefruit, 1978-79[z] (17).

Fumigation[y] Date	Ambient temperature (°C)	Cultivar	After 28 days in storage[x] Peel injury %	Decay %
Nov. 14	29	Ruby Red	0	3
		Marsh	0	0
Dec. 1	29	Thompson Pink	3	9
		Marsh	0	8
Jan. 26	10	Ruby Red	11	1
		Marsh	26	3
Feb. 6	20	Ruby Red	11	12
		Marsh	28	13
Feb. 14	16	Ruby Red	10	0
		Marsh	8	0
Feb. 20	19	Thompson Pink	10	0
Mar. 26	17	Ruby Red	0	0
Apr. 16	22	Thompson Pink	3	0

[z]Data for control fruit showed that no peel injury was present and decay did not exceed 5% at any insepction. Decay averaged less than 0.5% for all control fruit.

[y]On each date, cartons of fruit were fumigated with methyl bromide at application rates of 40 or 56 g/m^3 for 2 hr, with 20 and 80% load factors (3 and 12 cartons), respectively, in an 0.8 -m^3 chamber with continuous gas circulation. Pulp temperatures closely matched ambient temperatures.

[x]Cartons of fruit were stored at 16°C before January and 10°C thereafter to avoid chilling injury.

Table 3. Percentage irradiation injury immediately after removal from 28-day storage under optimum conditions[z](25).

		Dosage (krad) 0	7.5	15	30	60	90
Test	Date	(%)[y]	(%)	(%)	(%)	(%)	(%)
1	Oct. 81	0.0	—	—	25.9	43.1	60.9
2	Dec. 81	0.0	—	2.2	6.6	17.7	25.9
3	Feb. 82	0.2	—	0.2	3.9	26.2	39.7
4	Apr. 82	3.6	—	7.7	17.3	24.8	35.8
5	May 82	3.6	—	8.9	9.7	18.8	17.7
6	Sep. 82	0.0	0.0	0.0	0.2	1.1	0.0
7	Oct. 82	1.2	3.6	2.7	3.0	5.3	0.0

[z]Test 1 used a total of 320 fruit from 2 lots per irradiation level. All other tests used a total of 640 fruit from 4 lots per level, except for the 90-krad level in tests 6 and 7, where only 40 fruit were used per lot for a total of 160 fruit.

[y]Regardless of severity, all injury combined numerically to compute the percentage of injury.

adult each survived 15 and 30 krad treatments; both died before becoming sexually mature (36).

At present, high costs and lack of assurance concerning consumer acceptance are limiting factors to the use of gamma irradiation.

Cold treatment

Recommended storage temperatures for Florida grapefruit are 10°C for mid - and late-season fruit and 16° for early-season fruit. Grapefruit sustain chilling injury (CI) when exposed to temperatures below 10°, and the susceptibility of CI varies throughout the harvesting season (15, 33). Preharvest conditions in the grove, as well as postharvest handling, may directly affect the extent of CI in stored grapefruit (16). Pre - and postharvest applications of benomyl (41) and postharvest applications of thiabendazole (34) reduced CI. Waxing grapefruit and packaging in film minimized CI (14, 32, 40). Raising the relative humidity to 100% during storage (31), as well as intermittent warming (10, 27), greatly reduced CI.

Temperature preconditioning of grapefruit before low-temperature storage gave some success (15, 23). Constant storage at 1°C for 28 days resulted in excessive CI; however, preconditioning similar fruit for 7 days at 10°, 16° or 21° significantly reduced CI during 21 days of storage at 1° under high humidity conditions (19, 20), and this continued throughout the season for early, midseason and late grapefruit (Table 4). Degreening early grapefruit for long periods of time with ethylene tended to make the fruit more susceptible to CI (18).

Recent research showed that preconditioning grapefruit at 21° and 27°C for 7 days is significantly less effective than preconditioning for a similar period of time at 16° (21). Grapefruit infested with Caribbean fruit fly and stored for 14 days at 2° resulted in 100% mortality (5). Based on this research, the Government of Japan recently approved a cold treatment schedule for Florida grapefruit that is on a sliding scale beginning with 0.6°C for 10 days and extending to 2.2°C for 17 days with the stipulation that 1,500 fruit be held at 26.7°C for 10 days and then cut to determine presence of Caribbean fruit fly larvae. Although moisture loss is a contributing factor, it is noted that it does not appear to be the primary factor in CI (32). In Israel, recent studies showed that by combining the fungicide thiabendazole with cooling of grapefruit, susceptibility to CI can be reduced and the cold treatment can be practiced with a low CI risk (9).

During the 1981–82 season 4 relatively large-scale grapefruit tests were conducted at a commercial storage facility in Florida. Only 1% CI was observed for the entire season on fruit that had been preconditioned at 16°C for 7 days and stored at 1° for 21 days; a slight increase in CI occured during a 7-day holding period at 21° (Table 5). These tests were followed late in the 1982–83 season with an experimental shipment of 16,000 boxes of grapefruit to Japan (unpublished data). Sixty boxes of fruit representing 15 separate lots were retained in Tokyo for study; less than 0.4% of the preconditioned fruit showed CI while the fruit that was not preconditioned showed 2.2%. The following season, 1983–84, excessive losses were sustained in commercial ship-

Table 4. Chilling injury of preconditioned early, midseason, and late Florida grapefruit[z] (19).

Preconditioning and storage treatment[y] °C	Chilling injury after storage		
	Early (%)	Midseason (%)	Late[x] (%)
28 days at 1°	17.2a	17.7a	6.1a
28 days at 16°	0.0b	0.1b	0.0b
7 days at 16°+	-		
21 days at 1°	0.1b	0.6b	0.4b
28 days at 10°		0.1b	0.0b
7 days at 10°+			
21 days at 1°	-	0.9b	0.1b

[z] Each value represents 1,400, 1,320 and 720 fruit from early-, mid- and late-season harvests, respectively. Mean separation of columns by Duncan's multiple range test, 5% level.

[y] Relative humidity ranged from 88 to 92% for fruit at 1° to 10° C, and from 80 to 92% for those at 16°C.

[x] Controls for late fruit were held for 25 days instead of 28 and preconditioned fruit were held for 18 days instead of 21 days at 1°.

Table 5. Chilling injury of preconditioned Florida grapefruit in a large-scale commercial facility[z] (unpublished data).

Preconditioned and storage treatment[y] °C	Number of fruit	Chilling injury After storage (%)	Chilling injury After holding (7 days at 21°C) (%)
28 days at 1°	4,4125	14a[x]	17a[x]
28 days at 16°	4,431	0b	0b
7 days at 16°+ 21 days at 1°	13,232	1b	1b

[z]Each value represents 4 separate tests from 8 to 11 different lots. Tests were conducted in November 1981, December 1981, February 1982 and April 1982.

[y]Relative humidity ranged from 88 to 92% for fruit at 1°C and from 80 to 92% for those at 16°.

[x]Mean separation of columns by Duncan's multiple range test, 5% level.

ments to Japan. The cause for such losses cannot be fully explained. Proper temperatures and humidity levels were not maintained for the prescribed periods of time and this possibly relates to the large density and mass of thousands of boxes of fruit and the inadequate refrigeration equipment.

Additional research is needed to solve the problem. One approach will be to determine the safety of the fruit in van containers where a relatively small mass of approximately a thousand boxes of fruit is involved. Four citrus tests in California demonstrated that van containers maintained fruit with temperature uniformity throughout the load of 2.0 or 2.2°C for 14 days (28). Use of the cold treatment to Japan compared to EDB fumigation would result in treatment cost increases estimated at 25 to 40 cents per box.

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