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Cold Storage and Hot-water Immersion as Quarantine Treatments for Canistel Infested with Caribbean Fruit Fly

Guy J. Hallman
U.S. Department of Agriculture, Agricultural Research Service, Subtropical Horticulture Research Station, 13601 Old Cutler Road, Miami, FL 33158

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Abstract. **Canistel [*Pouteria campechiana* (HBK.) Baehni] fruit were subjected to cold storage and hot-water immersion treatments known to kill immature Caribbean fruit flies [*Anastrepha suspensa* (Loew)] in other fruit. Cold storage at 1 or 3C for 17 days did not cause appreciable loss in canistel quality compared with fruit stored at the normal 10C. Unripe canistels immersed in water at 46C for 90 min or at 48C for 65 min, however, developed dark blotches on the peel and a 2- to 3-mm-thick layer under the peel that did not soften. Canistels were infested with Caribbean fruit flies and subjected to 1 or 3C storage for up to 14 days. The resulting lethality data were fitted to three probability density functions (PDF) to estimate the number of days required to achieve quarantine security (99.9968% dead). The normal and Gompertz PDFs gave some reasonable estimates, while the logistic PDF gave low estimates. At 1C, 14 days would be needed to achieve quarantine security, while at 3C a minimum of 15 days would be required. These estimates must be tested to determine if they are valid after a large amount of Caribbean fruit fly immatures is subjected to the treatments.**

Canistel is an ovoid or top-shaped fruit 5 to 15 cm long with thin, yellow skin when ripe. The orange-yellow pulp is dry, mealy, mildly sweet, and has the consistency of hard-boiled egg yolk that gives the canistel the common name eggfruit. Besides being eaten fresh, canistels are used in pies, milk shakes, and ice cream. According to Morton (1987), the highly nutritious fruit, native from southern Mexico to El Salvador, deserves wider recognition. Canistels are harvested when the peel is beginning to turn yellow, as canistels harvested when green will not ripen properly. The harvest season in southern Florida is November to March. The canistel fruit is a host of the Caribbean fruit fly (Swanson and Baranowski, 1972), and, as such, the fruit must be subjected to quarantine treatment before they can be shipped to areas where this fly does not exist and might become established. I found no literature on quarantine treatments of canistels for any pest.

Recent reviews treat cold storage and hot water immersion as quarantine treatments (Gould, 1994; Sharp, 1994). Cold storage quarantine treatments for Florida grapefruit (*Citrus paradisi* Macf.) infested with Caribbean fruit fly vary from 14 days at 0.6C to 24 days at 2.2C (Ismail, 1989). Carambolas (*Averrhoa carambola* L.) infested with the Caribbean fruit fly are treated at 1.1C for 15 days (Gould and Sharp, 1990). Mangoes (*Mangifera indica* L.) infested with fruit flies in the Neotropics are immersed in water at 46.1 ± 0.05C for 75 to 90 min (Animal and Plant Health Inspection Service, 1990). Herein, I report on research using cold storage and hot-water immersion as potential quarantine treatments of canistels infested with Caribbean fruit fly immatures.

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Materials and Methods

Canistels (weight range, 67 to 422 g; mean, 186 g) were picked when they were beginning to turn yellow from trees on the U.S. Dept. of Agriculture (USDA), Subtropical Horticulture Research Station, in Miami.

Market quality of treated canistels. Cold storage treatments were done in walk-in coolers at 1, 3, and 10 ± 0.5C for 17 days. The canistels stored at 10C, which is commercial practice, were used as a control. This temperature is not expected to provide quarantine security against Caribbean fruit fly immatures (Benschoter and Witherell, 1984). Upon removal from cold storage, canistels were kept at 23 ± 1C until they ripened. The test was replicated four times with 12 canistels per treatment.

Canistels were treated with hot water in circulating water baths (model 260; GCA Corp. Chicago). Canistels initially at 24 ± 1C were immersed in water at 46 or 48C for 90 or 65 min, respectively. After treatment, half of the canistels were placed in 10C storage for 7 days and then at 23C until they were discarded. The other half were stored at 23C only. The test was replicated twice with 12 canistels per treatment. Number 36 gauge (0.127 mm in diameter) copper–constantin thermocouples were inserted to within 2 mm of the center of the canistels in the 46C treatments, and temperatures were recorded on a datalogger (model 2285B; John Fluke Manufacturing Co., Everett, Wash.) every minute.

Cold storage and hot-water immersion tests were followed by observations on peel color; ripening; decay; and pulp color, softness, texture, and flavor. Firmness was rated on a scale of 1 to 5: 1 = hard, no yield to moderately applied finger pressure (MAFP); 2 = fairly hard, slight yield to MAFP; 3 = fairly soft, moderate resistance to MAFP; 4 = soft-ripe, eating stage, little resistance to MAFP, peel may tear with MAFP; and 5 = overripe, mushy, very little resistance to MAFP. Peel color was rated on a 1 to 5 scale: 1 = green, no yellow color; 2 = yellow blush, 5% to 25% of surface area yellow; 3 = half yellow, 25% to 50% of surface area yellow; 4 = three-quarters yellow, 50% to 75% of surface area yellow; 5 = totally yellow. Blemishes developing on the peel surface were counted and measured. Pulp texture, color, and flavor were subjectively compared by me and a technician for differences between the treated canistels and the control (stored at 10C) canistels.

Lethality of cold treatment to Caribbean fruit fly immatures in canistels. Canistels were placed in an outdoor infestation cage (4 × 3 × 2.5 m) containing ≈200,000 Caribbean fruit flies, 85% of which were reared on an agar-based diet (wheat germ substituted for defatted wheat germ) with the remainder reared on a corncob-based diet (Burditt et al., 1975). The fruit were removed after 3 to 5 days and stored at 24 ± 1C for 2 to 3 days before being randomly divided into nine groups of equal numbers, four of which were placed in coolers at 1 or 3C for 2 to 14 days. The ninth group was placed over bins containing sand to recover larvae emerging from nontreated fruit that were used to estimate the number of immatures per treatment. The test was repeated three times using 36, 44, and 162 canistels per treatment level. Data were analyzed with the SAS Probit Procedure (SAS Institute, 1988). The normal, logistic, and Gompertz probability density functions (PDF) were used with and without log 10 conversion of days and with and without optimization of the natural death rate. No measure of the natural death rate was possible in these tests because the number of immatures per treatment was estimated after any natural death occurred. The normal PDF is most commonly used in analyzing dose-lethality data. All three PDFs give about the same responses in the range of lethal doses (LD) between LD_{10}–LD_{90} (10% to 90% lethality). Outside that range, however, estimates of lethality using the three PDFs can differ considerably. A quarantine treatment must meet probit 9 security (99.9968% lethality) to be accepted by the USDA, Animal and Plant Health Inspection Service (Chew and Ouye, 1985).

Results and Discussion

Market quality of treated canistels. After 17 days at 10C the canistels were slightly soft [mean firmness rating (MFR) = 2.6]. After an

additional 3 days at 23C all fruit were soft enough to eat (MFR = 4.2), and the peel was yellow [mean peel color (MPC) = 4.9] and slightly wrinkled. Canistels stored for 17 days at 1 or 3C remained firm (MFR = 2.1) and partially green (MPC = 3.4); after an additional 7 to 9 days at 23C these canistels were soft enough to eat (MFR = 4.3), and the peel was yellow (MPC = 4.9) and slightly wrinkled. When soft, the pulp of fruit subjected to all three treatments was orange-yellow, dry, mealy, and slightly sweet—conditions considered normal for ripe canistel. There was negligible decay until the canistels became overripe.

At the initiation of the immersion of canistels in 46C water, center temperatures were 25.2C. After 90 min, the centers of canistels that weighed ≤173, 196 to 212, 289 to 295, and 323 to 332 g were 45.9 to 46.0, 45.7 to 45.9, 45.3 to 45.5, and 44.8 to 45.0C, respectively. Within 3 days at 23C, canistels that had been immersed in water at 46C for 90 min or 48C for 65 min developed sunken, dark spots of 3 to 6 mm in diameter on the peel and remained undesirably firm to a depth of 2 to 3 mm below the peel surface. A corky layer developed underneath this firm layer (Fig. 1); below the corky layer the pulp softened normally. The canistels treated at 46 and 48C developed sunken, dark spots with a mean of 6.3 (range 0 to 18) and 14.3 (range 0 to 25) spots per fruit, respectively. One of 24 control canistels developed stem-end rot, while three and five of 24 had stem-end rot for the 46 and 48C treatments, respectively. Storage at 10C for 7 days immediately after hot-water immersion did not reduce damage to canistels caused by the treatment.

Lethality of cold treatment to Caribbean fruit fly immatures in canistels. About 2820 Caribbean fruit fly larvae were recovered from the three nontreated groups of canistels. In the first test, the estimated percentage of larvae killed (of 17 treated) during storage at 1C for 5, 7, 9, and 12 days was 94.1%, 94.1%, 100%, and 100%, respectively; at 3C the percentage killed was estimated to be 82.4%, 100%, 100%, and 100% for 5, 8, 12, and 14 days, respectively. In the second test, the estimated percentage of larvae killed (of 942 treated) at 1C was 14.5%, 8.0%, 92.8%, and 98.8% for 2, 4, 7, and 9 days, respectively; at 3C the percentage killed was 0%, 0%, 95.9%, and 99.3% for 3, 4, 7, and 11 days, respectively. For the third test, the estimated percentage of larvae killed (of 1861 treated) at 1C was 43.6%, 92.7%, 98.9%, and 99.9% for 3, 6, 8, and 10 days, respectively; at 3C the percentage killed was 45.7%, 90.8%, 99.4%, and 100% for 3, 6, 9, and 12 days, respectively. Estimates of 99.9968% lethality varied between 7.2 to 23.0 days (Table 1). Because of the lack of fit when all three tests were analyzed together, the third test, which yielded 66% of the insects, was analyzed separately. The estimates of the lethal time to 99.9968% lethality ($LT_{99.9968}$) mostly were slightly longer (7.5 to 30.8 days) (Table 2) than estimates in Table 1. Also, most of the results in Table 2 fit the PDFs used, whereas none in Table 1 fit. The $LT_{99.9968}$ estimates for the logistic PDF were illogically low given that larvae survived longer treatments than those predicted. This same phenomenon (underestimation of $LT_{99.9968}$ although the data contained survivors at treatment times longer than the estimated values) occurred to an even greater extent in cold treatment of white sapotes (*Casimiroa edulis* Llave & Lex.) infested with Caribbean fruit fly (Hallman, 1993). This result indicates that the logistic PDF should not be used to estimate $LT_{99.9968}$.

Benschoter (1983) achieved 100% kill of an estimated 34,100 Caribbean fruit fly eggs and larvae in grapefruit stored at 1.7C for 14 days. Gould and Sharp (1990) treated an estimated 105,100 Caribbean fruit fly larvae in carambolas at 1.1C for 13.6 days with no survivors, and achieved 99.93% Caribbean fruit fly kill following 1.1C for 12 days. Hallman (1993) achieved 99.99% Caribbean fruit fly larval kill in white sapotes stored at 1C for 11.5 days. Based on these data, it seems reasonable that the estimated $LT_{99.9968}$ for Caribbean fruit fly larvae in fruit at 1C would not exceed 13 to 14 days, unless fruit fly hosts affect the susceptibility of the larvae to cold

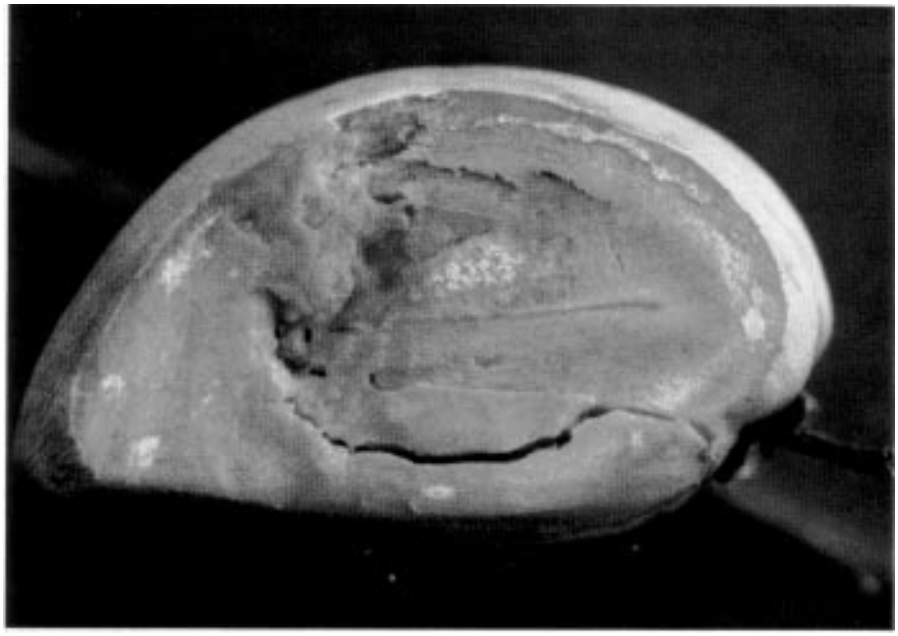

Fig. 1. Canistel that had been immersed in 46C water for 90 min within 24 h after harvest showing later development of corky layer beneath peel (light-colored patches) as canistel ripened.

Table 1. Analyses of death of Caribbean fruit fly infesting canistel stored at 1 or 3C using SAS Probit Procedure (SAS Institute, 1988); all three tests together.

Probability density function	Log 10 of days	Natural death optimized	$P > \chi^2$	Slope ± SEM	99.9968% death (95% FL), days[z]
Stored at 1C					
Normal	No	No	<0.01	0.53 ± 0.10	11.5 (9.3–17.2)
	No	Yes	<0.01	0.81 ± 0.46	10.0 (7.8–*)
	Yes	No	<0.01	5.4 ± 1.1	20 (12–78)
	Yes	Yes	<0.01	13.1 ± 4.2	10.5 (8.1–55)
Logistic	No	No	<0.01	0.93 ± 0.19	8.3 (6.8–12.2)
	No	Yes	<0.01	1.8 ± 0.77	7.4 (6.4–*)
	Yes	No	<0.01	9.3 ± 2.1	9.9 (7.0–27)
	Yes	Yes	<0.01	25 ± 9.0	7.6 (6.4–28)
Gompertz	No	No	<0.01	0.51 ± 0.12	12.8 (10–22)
	No	Yes	<0.01	0.47 ± 0.16	13.2 (10–35)
	Yes	No	<0.01	5.7 ± 1.1	23.0 (14–76)
	Yes	Yes	<0.01	8.1 ± 0.17	16.4 (10–*)
Stored at 3C					
Normal	No	No	<0.01	0.57 ± 0.16	11.3 (8.5–23)
	No	Yes	<0.01	0.93 ± 1.1	9.5 (6.3–*)
	Yes	No	<0.01	6.2 ± 1.2	17.7 (11–59)
	Yes	Yes	<0.01	14.0 ± 14	10.1 (6.1–*)
Logistic	No	No	<0.01	1.0 ± 0.22	8.2 (6.8–12.5)
	No	Yes	<0.01	2.1 ± 3.0	7.2 (*)
	Yes	No	<0.01	10.9 ± 2.4	9.5 (6.9–22)
	Yes	Yes	<0.01	29 ± 13	7.3 (6.2–*)
Gompertz	No	No	<0.01	0.50 ± 0.31	13.1 (8.2–*)
	No	Yes	<0.01	0.46 ± 0.36	13.6 (8.0–*)
	Yes	No	<0.01	6.5 ± 1.7	20.3 (12–187)
	Yes	Yes	<0.01	8.2 ± 10	16.3 (7.8–*)

[z]FL (fiducial limits) represented by asterisk (*) not estimable because of poor fit.

Table 2. Analyses of death of Caribbean fruit fly infesting canistel stored at 1 or 3C using SAS Probit Procedure (SAS Institute, 1988); test 3 only.

Probability density function	Log 10 of days	Natural death optimized	$P > \chi^2$	Slope ± SEM	99.9968% death (95% FL), days[z]
Stored at 1C					
Normal	No	No	0.081	0.51 ± 0.02	11.0 (10.4–11.8)
	No	Yes	<0.01	0.50 ± 0.06	11.1 (9.8–13.2)
	Yes	No	0.036	5.6 ± 0.25	16.4 (14.4–19.4)
	Yes	Yes	0.39	7.8 ± 0.79	13.7 (12.3–16.1)
Logistic	No	No	0.43	0.95 ± 0.03	7.5 (7.3–7.7)
	No	Yes	0.34	1.06 ± 0.11	7.6 (7.3–7.9)
	Yes	No	<0.01	10.3 ± 0.92	7.8 (6.7–9.6)
	Yes	Yes	0.15	17.9 ± 1.7	7.8 (7.5–8.3)
Gompertz	No	No	<0.01	0.43 ± 0.14	13.3 (9.7–35)
	No	Yes	<0.01	0.41 ± 0.19	13.7 (9.4–*)
	Yes	No	0.45	4.9 ± 0.12	25.0 (23.1–27.1)
	Yes	Yes	0.012	4.8 ± 0.56	25.9 (19.6–42)
Stored at 3C					
Normal	No	No	0.11	0.46 ± 0.01	11.9 (11.5–12.4)
	No	Yes	<0.01	0.45 ± 0.06	12.0 (10.5–14.5)
	Yes	No	<0.01	5.1 ± 0.30	19.3 (15.9–24.7)
	Yes	Yes	0.39	7.5 ± 0.71	14.9 (13.3–17.7)
Logistic	No	No	0.36	0.84 ± 0.03	8.0 (7.7–8.3)
	No	Yes	0.39	0.97 ± 0.10	8.1 (7.8–8.4)
	Yes	No	<0.01	9.3 ± 1.0	8.5 (7.1–11.5)
	Yes	Yes	0.21	17.2 ± 1.6	8.3 (7.9–8.9)
Gompertz	No	No	<0.01	0.39 ± 0.07	14.4 (11.7–20.3)
	No	Yes	<0.01	0.36 ± 0.12	15.0 (10.9–46)
	Yes	No	0.74	4.5 ± 0.12	30.2 (27.5–33.5)
	Yes	Yes	0.16	4.4 ± 0.26	30.8 (26.6–37)

[z]FL (fiducial limits) represented by asterisk (*) not estimable because of poor fit.

storage. Given the high death rate of Caribbean fruit fly larvae in canistels at 1C in this study (99.946% after 10 days), canistels do not appear to increase cold tolerance of Caribbean fruit fly compared with carambolas and white sapotes. Therefore, estimates of $LT_{99.9968}$ provided by the Gompertz PDF (without log 10 of days at 1C) and the normal PDF with log 10 of days at 1C (Table 2) appeared to be the most reasonable of the analyses used. However, fit to the Gompertz PDF was poor, resulting in high upper fiducial limits. These same observations were made for Caribbean fruit fly death in white sapotes held at 1C (Hallman, 1993). In any case, for 1C to be approved as a quarantine treatment for canistels, a large number of Caribbean fruit fly immatures must be killed during a given period with no survivors (confirmatory test) (Chew and Ouye, 1985). Based on the results of this study and previous research with cold-storage quarantine treatments of fruit infested with Caribbean fruit fly (Benschoter, 1983; Gould and Sharp, 1990; Hallman, 1993), I recommended that 14 days be attempted for the confirmatory test with canistels. Because the canistel acreage in southern Florida is small, a significant portion of the harvest would need to be dedicated to this test for 2 to 3 years to accomplish it.

$LT_{99.9968}$ estimates at 3C were slightly longer than $LT_{99.9968}$ estimates at 1C (Tables 1 and 2). A quarantine treatment could probably also be developed at 3C for canistels infested with Caribbean fruit fly. A confirmatory test at 3C should be attempted for a minimum of 15 days.

An additional benefit from this research is that canistels may be stored at temperatures <10C, as presently used, which may greatly extend the storage life of this seasonal fruit.

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