Table 1. Probit analysis on log10 of time (minutes) of hot water dip.

Treatment	43.3°C hot water dip no cold treatment	43.3°C hot water dip one week at 1.1°C
LD50	42.8 minutes	29.3 minutes
LD90	67.3 minutes	45.9 minutes
LD99	97.2 minutes	66.3 minutes
Probit 9 (LD99.9968)	175.5 minutes	100.2 minutes

highly divergent (Table 1). As the dosage (length of hot water immersion) is increased from one required to achieve LD99 to the dosage required to achieve Probit 9, the time gap widened between the two treatments. There was a difference of 75 minutes between the two treatments at Probit 9. Combining a cold treatment with the hot water immersion cut the hot water immersion time required to reach Probit 9 almost in half. This may allow a hot water immersion treatment which will not harm the grapefruit, yet allow the treatment to be completed in less time than the current standard cold storage quarantine treatments.

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PHYTOTOXICITY TO GRAPEFRUIT EXPOSED TO HOT WATER IMMERSION TREATMENT

W. R. MILLER, R. E. MCDONALD AND T. T. HATTON
U.S. Department of Agriculture, ARS
2120 Camden Rd., Orlando, FL 32803

M. ISMAIL
Florida Department of Citrus
700 Experiment Station Road
Lake Alfred, FL 33850

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Abstract. **The effect of immersing grapefruit (*Citrus paradisi* Macf.), cv. Marsh, for 4.5 hr in 43.5°C (110°F) water is reported to provide effective quarantine protection against the Caribbean fruit fly (*Anastrepha suspensa* Loew). Exposure of freshly harvested grapefruit to this hot water, time/temperature regime was phytotoxic to the peel. Phytotoxicity was expressed as peel discoloration, puffiness, and decreased resistance of peel to penicillium infection after treatment and storage. Fruit subjected to the hot water treatment increased 1% in volume during the treatment compared to little or no change for those in ambient water or nonimmersed control fruit. After 2 weeks' storage at 10°C (50°F), hot water-treated fruit had about 45% decay compared to 6% and 1% in fruit immersed in ambient water or nonimmersed fruit, respectively. Hot water-immersed fruit were significantly more deteriorated than those of other treatments based on condition of the stem scar, appearance of the peel, and fruit firmness. Based on observations of late-season fruit in 3 separate tests during the 1987/88 season, we concluded that a 4-hr hot water treatment at 43.5°C was too phytotoxic to serve as a quarantine treatment.**

Grapefruit (*Citrus paradisi* Macf.) exported from Florida to certain countries, such as Japan, must be certified as free from the Caribbean fruit fly (*Anastrepha suspensa*). Certification may be accomplished by harvesting fruit from certified fly-free zones, or by subjecting fruit to the approved cold treatment procedure (1). Since November 30, 1987, when ethylene dibromide (EDB) fumigation of grapefruit was suspended by the Japanese government, investigations for alternative methods to control the Caribbean fruit fly have intensified.

Currently, hot water treatment is approved as a quarantine treatment for the control of the West Indian (*A. obiqua*) and Caribbean fruit flies in mango (cv. Francis) imported into the United States from Haiti (1). For papayas, the combination of hot water and EDB fumigation or a double hot water treatment are approved quarantine procedures (1). The Plant Protection and Quarantine Treatment Manual also lists a vapor heat treatment for the

control of the Mexican fruit fly (*A. ludens*) for grapefruit, orange, tangerine, and mango. Hot water has been investigated as a quarantine procedure for control of various insects in banana (2), papaya (4, 5, 6), peach (7), and mango (8, 10, 12, 13). However, there is no approved hot water immersion treatment for the control of the Caribbean fruit fly in Florida grapefruit. In 1963, Smoot (14) investigated hot water as a fungicidal treatment for Florida round oranges. He found that 53°C (127°F) water for a 5-min duration was effective for decay control; however, for quarantine purposes, exposure time is usually longer than used for decay control.

Recently, Sharp (9) found that 49°C (120°F) hot water treatment for 10-40 min caused grapefruit peel damage, but indicated that fruit exposed to 40-43°C (104-109°F) water for 6 to 8 hr might kill infestations of Caribbean fruit fly without fruit damage. Sharp further determined time-mortality correspondence of various life cycle stages of the Caribbean fruit fly when exposed to 43.3°C water (11). Exposure for about 26 min is required for probit 9 mortality by this model. Sharp, (personal communication, U.S.D.A. Subtropical Horticultural Research Laboratory, Miami, FL) indicated control of the Caribbean fruit fly might be achievable at probit 9 in grapefruit after immersion for about 4.5 hr in 43.5°C water. Since fruit were severely desiccated during the infestation and incubation period required for entomological testing, it was not possible to evaluate phytotoxic effects of this treatment on sound grapefruit. Therefore, the purpose of this report is to describe the phytotoxic effects on freshly harvested grapefruit following exposure for 4 hr to 43.5°C water and subsequently stored for 3 weeks at 10°C (50°F) plus 1 week at 21°C (70°F).

Materials and Methods

For this study, 'Marsh' grapefruit, size 40 count, were obtained from the Indian River region of Florida. The experiment was replicated 3 times with fruit harvested on 23 March, 30 March and 6 April 1988. All fruit were picked up at the packinghouse and held overnight at 26.8°C (80°F). For each of the 3 tests, 135 fruit were randomly divided, 45 fruit each for 3 treatments. In addition, the volumes of 20 fruit (10 each for the hot and ambient water treatments) were determined before and after treatment. The 3 treatments were: 1) immersion of fruit in 43.5°C water for 4 hr, 2) immersion of fruit in ambient water (about 24°C) for 4 hr, and 3) fruit were held in ambient air for 4 hr. During treatment, pulp temperatures were measured at 15-min intervals by thermocouples placed at the center of fruit. After treatment, fruit were washed and waxed (FMC Corporation Flavor Seal 93), but no fungicide was applied. Forty-five fruit of each treatment were divided into three 15-fruit subsamples and placed into separate 2/5-bu. citrus boxes. Fruit were stored for 3 weeks at 10°C plus 1 week at 21°C and inspected weekly. At each inspection, fruit were evaluated for soundness, i.e. aging, pitting, scalding, and for decay, firmness (subjectively), condition of rind and stem scar.

Data of fruit condition were subjected to ANOVA procedures, and Duncan's new multiple range test to determine differences among treatments.

Results

The hot water bath for the 3 tests averaged 45°C (113°F) at the beginning of fruit treatment and was 43.5°C (110 °F) within 1 hr lapse time. Average pulp temperature

Table 1. Percentage of sound, aged, pitted, scald and decayed fruit treatment and storage of 1, 2, and 3 wk at 10°C plus 1 wk at 21°C, averaged over 3 tests.

Storage time/ treatment	Sound	Age	Pit	Scald	Penicillium rot	Total decay
Initial[z]						
Water 43.5°C	100.0 a[y]	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Water 24.0°C	100.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Air	98.5 a	1.5 a	0.0 a	0.0 a	0.0 a	0.0 a
1 Wk 10°C						
Water 43.5°C	96.3 a	0.7 a	1.5 a	1.5 a	0.0 a	0.0 a
Water 24.0°C	97.0 a	0.0 a	1.5 a	0.0 a	0.0 a	1.5 a
Air	98.5 a	1.5 a	0.0 a	0.0 a	0.0 a	0.0 a
2 Wk 10°C						
Water 43.5°C	43.7 a	0.7 a	3.7 b	6.7 a	44.4 b	45.2 b
Water 24.0°C	87.5 b	1.5 a	1.5 ab	3.7 a	2.2 a	5.9 a
Air	94.8 b	4.4 a	0.0 a	0.0 a	0.7 a	0.7 a
3 Wk 10°C						
Water 43.5°C	34.3 a	1.0 a	1.9 a	13.3 b	48.6 b	49.5 b
Water 24.0°C	82.8 b	3.7 a	0.7 a	5.2 ab	4.4 a	8.1 a
Air	87.4 b	9.6 a	1.5 a	0.0 a	0.7 a	1.5 a
Plus						
1 Wk 21°C						
Water 43.5°C	36.7 a	0.0 a	0.0 a	4.4 a	53.3 b	56.7 b
Water 24.0°C	81.1 b	10.0 a	1.1 a	1.1 a	6.7 a	6.7 a
Air	71.1 b	21.1 a	1.1 a	1.1 a	3.3 a	5.6 a

[z]Immediately after treatment.
[y]Values in columns by inspection group followed by different letters are significantly different by Duncan's multiple range test at the 5% level of significance.

at the center of the hot-water-treated fruit for 3 tests was 43.5°C (110°F) after 2-hr and 15-min lapse time. Water temperature of the ambient bath was 23.9°C (75°F, ±1) during the 4-hr treatment and temperature of the ambient air was 24.4°C (76°F) over the 3 tests. Pulp temperatures of fruit in both ambient water and ambient air treatments were equal with their environment within 1 hr of start of treatment.

Hot-water-treated fruit increased in volume by 1.2% during treatment compared to –0.03% or no change for fruit held in water at ambient temperature ($p < 0.05$). Fruit held in ambient air during the 4-hr treatment was not volumetrically measured and assumed not to change.

The detrimental effects of the hot water treatment are shown in Table 1. After 2 weeks of storage, the percentage of sound fruit was about 44% for hot-water-treated fruit compared to 88 and 95% for fruit treated in ambient water or air, respectively. At this same inspection, decay in hot-water-treated fruit was 7.5 times higher than decay found in fruit treated in ambient water and about 60 times that of fruit held in ambient air. There was more pitting and a tendency for hot-water-treated fruit to have more scald compared to other treatments. After the third week of storage, the incidence of scald in fruit treated with hot water was 2.5 times higher than that in either ambient water or air-treated fruit. The relative incidence of decay and sound fruit was similar to that observed after 2 weeks' storage. After the third week of storage, all hot water-treated fruit in test 1 (23 March lot) were decayed and fruit of test 1 was terminated after 3 weeks' storage.

After fruit of test 2 and 3 (30 March, 6 April) were held 1 additional week at 21°C, the percentage of sound fruit decreased to about 37% for those treated in hot water and was 81 and 71% for those treated in ambient water or air, respectively. At this final inspection, average decay increased to 57% and the relative difference in percentage of decay among treatments remained the same as that found in the 2 previous inspections. Inconsistent differences in results after the final inspection compared to earlier inspections are influenced by the early storage termination of fruit in the March 23 test.

In addition, the higher incidences of decay in fruit treated in hot water probably concealed the visible symptoms of pitting and scald compared to those observed in fruit of other treatments. It is of interest to note that fruit held in ambient air during the 4-hr treatment tended to have higher incidences of aging than fruit of other treatments. Fruit held in water probably had a higher peel moisture content at the start of storage than fruit held in air. Similar reductions in aging were reported by Albrigo, et al. for oranges treated with preharvest antitranspiration compounds (3).

Average fruit firmness and freshness during storage by treatment are shown in Table 2. Immediately after storage, hot-water-treated fruit were softer than those of the other treatments. Hot-water-treated fruit changed little in firmness over storage duration, but were generally softer at each inspection than either ambient-water- or air-treated fruit. The rind and stem scar of hot-water-treated fruit were less fresh in appearance than those for fruit of other treatments after 3 weeks of storage. After the final inspection, hot-water-treated fruit were less firm and the peel less fresh in appearance than for other treatments.

The hot water treatment of 43.5°C for 4-hr duration is phytotoxic to freshly harvested grapefruit. Hence, we do not recommend this treatment for application as a quarantine procedure for the control of the Caribbean fruit fly. Since the core temperature of fruit reached 43.5°C in about 2 hr after the initiation of the hot water treatment, there may be alternative combination treatments of different time durations, temperatures and/or hot and cold water, or air temperatures which may provide control of this pest, but which are not phytotoxic to grapefruit. Therefore, we would encourage continued investigation to seek methods utilizing hot water, liquid or vapor, which will successfully control this fly without damaging the fruit.

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Table 2. Average index values for fruit of 3 harvest dates for firmness, peel and stem scar freshness of grapefruit after treatment and storage.

	Fruit condition indices								
	Firmness[z]			Rind[y]			Stem scar[x]		
Treatment[w]	1	2	3	1	2	3	1	2	3
Inspection									
Initial	2.7 b[v]	1.8 a	1.9 a	1.4 a	1.0 a	1.1 a	1.4 a	1.0 a	1.1 a
1 Wk 10°C	2.4 b	1.9 ab	1.8 a	1.8 a	1.4 a	1.3 a	2.1 a	1.3 a	1.7 a
2 Wk 10°C	2.4 a	2.0 a	1.9 a	2.4 a	1.8 a	1.4 a	2.4 a	1.7 a	1.7 a
3 Wk 10°C	2.5 b	2.0 a	1.9 a	2.6 b	2.1 a	2.0 a	3.0 b	2.4 ab	1.9 a
Plus									
1 Wk 21°C	2.7 b	2.3 ab	2.0 a	2.8 b	2.3 a	2.2 a	3.0 a	2.7 a	2.5 a

[z]Firmness index: 1 = firm, 2 = fairly firm, 3 = soft.
[y]Rind index: 1 = fresh, 2 = fairly fresh, 3 = old.
[x]Stem scar index: 1 = fresh, 2 = fairly fresh, 3 = old.
[w]Treatment 1 = 43.5°C water, 4 hr; treatent 2 = 23°C water, 4 hr; treatment 3 · ambient air, 4 hr.
[v]Values in rows by fruit condition groups followed by different letters are significantly different by Duncan's multiple range test at the 5% level of significance.

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STATUS OF HOT WATER IMMERSION QUARANTINE TREATMENT FOR TEPHRITIDAE IMMATURES IN MANGOS

JENNIFER L. SHARP
USDA, ARS, Subtropical Horticulture Research Station
13601 Old Cutler Road
Miami, Florida 33158

Additional index words. fruit fly, Probit 9, quarantine security, *Ceratitis, Anastrepha.*

Abstract. **Research performed by the Agricultural Research Service (ARS) led to approval by the Animal and Plant Health Inspection Service (APHIS) of hot water immersion quarantine treatments for mangos infested with fruit fly immatures. Thus, 'Francis' mangos from Haiti and mangos from Mexico that have been treated with hot water currently are imported into the United States. Similar treatments that disinfest fruit fly immatures in mangos are expected to be recommended by ARS and approved by APHIS for Puerto Rico and Peru. Hot water immersion quarantine treatment research is also underway in Brazil, Jamaica, Venezuela, and Guatemala.**

Mangos, *Mangifera indica* L., imported into the United States from countries having fruit fly pests are subjected to federal quarantine regulations. Hot water immersion was approved by the Animal and Plant Health Inspection Service (APHIS) to disinfest Haitian 'Francis' mangos having immatures of the West Indian fruit fly, *Anastrepha obliqua* (Macquart) and Caribbean fruit fly, *A. suspensa* (Loew) (1) and for Mexican mangos infested with Mexican fruit fly, *A. ludens* (Loew) and *A. obliqua* (2). Hot water quarantine treatments were recommended by the Agricultural Research Service (ARS) for Florida mangos infested with *A. suspensa* and mangos from the state of Chiapas, Mexico infested with the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and the so-called dark fruit fly, *A. serpentina* (Wiedemann) (16, 18). Approved hot water treatments have stimulated work in Caribbean and Central and South American countries which grow mangos and have fruit flies, as growers desire to export mangos to the United States.

Herein is presented summary data obtained from research performed since 1984 that provides the status of hot water immersion as a quarantine treatment against Tephritidae immatures in mangos.

Materials and Methods

All tests were done with 'Francis,' 'Oro,' and 'Ataulfo' mangos (individual weights of 350-590 g, 0.8-1.3 lb.), and 'Tommy Atkins,' 'Keitt,' and 'Haden' mangos (individual weights of 450-700 g, 1-1.54 lb.). Procedures required to perform all tests were reported by Sharp et al. (15, 16, 17, 18). Briefly, procedures are discussed as follows. Wild strains of fruit flies were collected as larvae from host fruits, and laboratory strains were reared from artificial diets (4, 7, 8, 10, 12). Female flies oviposited in mangos in cages. Infested mangos were cleaned, randomized to insure similar infestation levels per mango, and held for several days so larvae could develop to late second and third instars, the stages at which they move the greatest distance into the pulp. Infested mangos were divided into groups of equal numbers, put into mesh sacks, and immersed in circulating water at 46.1 ± 0.5°C in metal containers (13) for 10-80 min to obtain laboratory data to estimate Probit 9 security (99.9968% mortality) (3). Equal numbers of infested mangos not immersed were the controls used to estimate the number of treated larvae. Treated and nontreated mangos were held in separate racks over sand (5). The sand below each rack was sifted 2-3 times each week for several weeks to recover all larvae and pupae. Also, mangos held over strainers were sprayed with water to recover all immatures that remained in the fruits (17). The number of normally formed pupae was used in the data analysis. Data were analyzed to the 99% mortality level with the probit and GLM procedures of the Statistical Analysis Systems (SAS) (9). The fiducial limits for immersion times corresponding to 99.9968% mortality were obtained by using the formula in Finney (6). Pupae were held for eclosion, and the number of flies that emerged was recorded.

Results of research only are reported and mention of a trade name does not constitute a recommendation by the U.S. Dept. of Agriculture.