Immersion of Florida Mangos in Hot Water as a Quarantine Treatment for Caribbean Fruit Fly (Diptera: Tephritidae)

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ABSTRACT Heated water was tested as a quarantine treatment to destroy all instars of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), in mangos, *Mangifera indica* L., from Florida. Infested 'Tommy Atkins' and 'Keitt' were immersed for 20-60 min in water at 46.1-46.7°C. Probit analysis of the data estimated the immersion time required to reach 99.9968% mortality (Probit 9 security) as 60 and 60.5 min for 'Tommy Atkins' and 'Keitt,' respectively. A large-scale test resulted in zero survivors based on the number of normal appearing pupae when 116,031 *A. suspensa* larvae in 3,828 infested 'Tommy Atkins,' 'Keitt,' 'Jubilee,' and 'Kent' were immersed in water at 46.1-46.7°C for 90 min.

KEY WORDS Insecta, *Anastrepha suspensa, Mangifera indica,* quarantine security, plant quarantine

MANGOS, *Mangifera indica* L., are subjected to federal and state quarantine regulations because of possible infestations by tephritid fruit flies (Animal and Plant Health Inspection Service 1985). The Environmental Protection Agency banned the use of ethylene dibromide (EDB) on all fruit intended for consumption in the United States effective 30 September 1987 (Anonymous 1986). After September 1987, mangos from Florida could not be exported to Japan, Hawaii, California, Arizona, or Texas because there was no alternative quarantine treatment for EDB to destroy possible infestations of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Swanson & Baranowski 1972).

Immersion of infested Florida mangos in water at 46.1-46.7°C destroys eggs and larval (1-2 d old) infestations of *A. suspensa,* does not adversely affect fruit quality, and is a promising alternative quarantine treatment to EDB (Sharp & Spalding 1984, Sharp 1986, Spalding et al. 1988). However, before an effective quarantine treatment by regulatory agencies can be accepted, research must demonstrate that all larval instars present in the fruit at harvest would be killed by a specified treatment. Here we report results of studies on the efficacy of heated water as a quarantine treatment to kill second- and third-instar *A. suspensa* in Florida mangos.

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

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Materials and Methods

'Tommy Atkins' and 'Keitt' mangos (size 8 or smaller, equal to 8 mangos per box with a net weight of 5 kg and an average weight of 625 g, but not >700 g each) were infested for 2-6 d in separate, large outdoor cages each containing approximately 200,000 laboratory-reared *A. suspensa* (Burditt et al. 1974). Fruits were then removed from the cages, held at 22-24°C until the ninth day after fruits were put into the infestation cages, and immersed in water at 46.1-46.7°C to treat larvae. Outdoor ambient temperature and RH ranged from 23 to 32°C and 65 to 100%. Under laboratory conditions at 26 ± 2°C, 60 ± 5% RH, and a photoperiod of 14:10 (L:D), *A. suspensa* eggs hatch in 3 d, and the first, second, and third instars last 2, 2, and 3 d, respectively (Lawrence 1979). Eggs hatch in 57 h if held at 30°C in the laboratory (Prescott & Baranowski 1971).

The fruits of each cultivar were divided into lots of equal numbers. The lot randomly selected from the total (for each cultivar) and held as an untreated control was used to estimate the number of survivors present in the fruit at the time of treatment, because the number of survivors in treated fruit cannot be measured directly. The other lots were immersed in water at 46.1-46.7°C for 20, 30, 40, 50, or 60 min and replicated seven or eight times at each time for each cultivar to acquire the time-mortality data required to estimate 99.9968% mortality (Probit 9; a value currently used as a measure of quarantine security) for larvae. Infested fruit were immersed in heated water as described previously (Sharp 1986).

Table 1. Time–mortality relationships for *A. suspensa* larvae 1–6 d old in 'Tommy Atkins' submerged in water at 46.1–46.7°C

Time (t, min)	Treated larval population	No. pupae recovered (no. adults eclosed)	Mortality %
20	3,748	1,012 (395)	73.00
30	3,748	278 (121)	92.60
40	3,748	42 (24)	98.90
50	3,748	1 (1)	99.97
60	3,748	0	100.00

Estimated by SAS probit analysis: mortality (in probits) = 3.9126 + 0.0848t; SE (slope) = 0.0025 and SE (intercept) = 0.0637.

Table 2. Time–mortality relationships for *A. suspensa* larvae 1–6 d old in 'Keitt' submerged in water at 46.1–46.7°C

Time (t, min)	Treated larval population	No. pupae recovered (no. adults eclosed)	Mortality %
20	5,554	1,470 (639)	73.5
30	5,554	442 (178)	92.0
40	5,554	53 (28)	99.0
50	5,554	3 (2)	99.95
60	5,554	0	100.00

Estimated by SAS probit analysis: mortality (in probits) = 3.9459 + 0.0835t; SE (slope) = 0.0020 and SE (intercept) = 0.0520.

Untreated and treated fruit were placed in holding cages over sand at ambient temperatures (22–27°C) (Burditt & von Windeguth 1975). Sand beneath each cage was sifted three to four times per week for 5 wk to recover all surviving larvae and pupae. All survivors were held for eclosion at 24–27°C in gauze-covered plastic cups. For analysis, normally formed puparia were considered survivors.

Data were analyzed with the probit and GLM procedures of the Statistical Analysis System (SAS) (SAS Institute 1985). The fiducial limits for the immersion time corresponding to 99.9968% mortality (Probit 9) were obtained by using the formula in Finney (1980).

Based on laboratory tests of other USDA–ARS research and the need to assure quarantine security if other *Anastrepha* species or *Ceratitis* invade Florida, we selected an excessively high treatment time. The large-scale test allows no more than 32 survivors out of 1 million individuals treated (Baker 1939) at the upper fiducial limit. Replicated tests (10) were conducted July–August 1987 with 'Tommy Atkins,' 'Keitt,' 'Jubilee,' and 'Kent' (size the same as described previously) infested for 2–4 d and treated when larvae were 2–6 d old. One-tenth to 25% of the infested fruits was used as the untreated control, and the rest were immersed in water at 46.1–46.7°C for 90 min. Treated and control fruits were held in cages kept in separate buildings to avoid contamination. Sand in trays below each cage was sifted three to five times each week for 5 wk and examined for larvae or pupae. The criterion for effectiveness of the treatment was based on the number of normally formed puparia.

Results and Discussion

The study of 'Tommy Atkins' done during June–July 1986 and 1987 was based on a total of 828 fruit and an estimated treated fly population of 18,740 (Table 1). From the probit equation, the estimated submersion times for 50, 90, 95, 99, and 99.9968% mortality were 12.8, 27.9, 32.2, 40.3, and 60 min, respectively. For 99.9968% mortality, the estimated 95% upper and lower fiducial limits were 62.1 and 58.1 min, respectively. Percent eclosion of flies from pupae collected from the untreated fruit was 81. The study of 'Keitt' was conducted during July–August 1986 and 1987 with a total of 490 fruit and an estimated fly population of 27,740 (Table 2). The projected submersion times for 50, 90, 95, 99, and 99.9968% mortality were 12.6, 28, 32.3, 40.5, and 60.5 min, respectively. Percent eclosion of flies from pupae collected from the untreated fruit was 86. A total of 116,031 late second and third instars was estimated killed when 3,828 infested fruits were immersed in water at 46.1–46.7°C for 90 min (Table 3). Because no survivors were found of 116,031 larvae treated, we have 95% confidence that the true (unknown) survival rate of the treatment is <26 per million (Couey & Chew 1986). The average number of larvae recovered per control fruit was 30.3 (range, 1.4–69.4), and the average percent of flies that emerged from the pupae (control) was 78.3%.

The hot-water treatment described herein appears to be a preferred quarantine treatment for mangos compared with fumigation with methyl bromide. 'Keitt' and 'Tommy Atkins' fumigated with 32 and 48 mg/liter, respectively, for 2 h at 21°C show increased development of stem-end rot and anthracnose decay and severe pitting of hard green mature fruit compared with nonfumigated mangos (Spalding et al. 1977). Another possible quarantine treatment is irradiation of infested fruit. At high doses of 25 to 150 krads, which are above doses required to kill fruit fly immatures, irradiation of 'Tommy Atkins' and 'Keitt' produces scald-like browning of the skin and dark sunken areas (Burditt et al. 1981). Immersion of 'Tommy Atkins' and 'Keitt' in water at 46.1–46.7°C for 90 min was shown to be an effective quarantine treatment method that destroyed *A. suspensa* larvae in Florida mangos in this study. Also, the treatment did not cause damage to the quality of the fruit (Sharp & Spalding 1984, Spalding et al. 1988). The data obtained from this study, including the large number of larvae killed, are required for the approval of a hot-water treatment certification schedule for *A. suspensa* in Florida mangos.

Table 3. Summary of large-scale test. Confirmation of estimated Probit 9 security (99.9968% mortality)

Infestation date	Treatment date	No. non-treated mangos (control)	No. treated mangos (cultivars)	Estimated no. larvae treated	Age (d) of treated larvae (instars)[a]
29 June–2 July	8 July	58	174 ('Tommy Atkins')	249	3–6 (II, III)
6–9 July	15 July	44	396 ('Tommy Atkins')	7,947	3–6 (II, III)
7–10 July	16 July	43	387 ('Tommy Atkins')	5,607	3–6 (II, III)
13–17 July	22 July	66	594 ('Tommy Atkins')	11,250	2–6 (I, II, III)
18–21 July	27 July	45	405 ('Tommy Atkins')	5,374	3–6 (II, III)
20–22 July	29 July	48	432 ('Tommy Atkins')	25,214	4–6 (II, III)
21–23 July	29 July	45	405 ('Tommy Atkins')	28,089	3–6 (II, III)
25–28 July	3 Aug.	37	333 ('Keitt')	16,299	3–6 (II, III)
27–30 July	4 Aug.	42	378 ('Jubilee,' 'Kent')	9,819	2–4 (I, II)
28–31 July	5 Aug.	36	324 ('Kent')	6,183	2–5 (I, II, III)
Total		464	3,828	116,031	

[a] Larval instar was calculated if eggs hatched in 3 d after the method of Lawrence (1979).

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