Mortality of Caribbean Fruit Fly (Diptera: Tephritidae) Larvae Infesting Mangoes Subjected to Hot-Water Treatment, then Immersion Cooling

GUY J. HALLMAN AND JENNIFER L. SHARP

Subtropical Horticulture Research Station, USDA-ARS, Miami, Florida 33158

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ABSTRACT Cooling mangoes after their immersion in 46.1–46.7°C water was evaluated for its effect on mortality of Caribbean fruit fly, *Anastrepha suspensa* (Loew), larvae. More time to achieve Probit 9 (99.9968%) mortality was estimated for larvae in mangoes that were allowed to cool slowly in relatively still air at 23 ± 0.5°C, compared with larvae in mangoes that were cooled rapidly in circulating water at 21 or 27 ± 0.5°C. However, the differences were not statistically significant. One larva of *A. suspensa* survived out of an estimated 17,589 larvae infesting mangoes that were immersed in 46.1–46.7°C water for 54 min and then immediately placed in 21 ± 0.5°C water. No survivors were found when infested mangoes containing an estimated 17,589 larvae were immersed in 46.1–46.7°C water for 54 min and cooled in 23 ± 0.5°C air. These results suggest that the effects of rapidly cooling commodities after they are subjected to heat quarantine treatments should be considered when the treatments are developed, especially if rapid cooling of the commodities might be practiced.

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

HOT-WATER IMMERSION was approved by the USDA, Animal and Plant Health Inspection Service (1987) for quarantine treatment of 'Francis' mangoes (*Mangifera indica* L.) and similar cultivars from Haiti that are hosts of the West Indian fruit fly (*Anastrepha obliqua* (Macquart)) and Caribbean fruit fly (*A. suspensa* (Loew)) (Anonymous 1990). Approval of the treatment required that at least 93,613 larvae of each species in mangoes be subjected to hot-water immersion with no survivors (Couey & Chew 1986). The treatment does not restrict cooling mangoes after hot-water immersion; packers wish to cool fruits immediately after heating to delay ripening and retard decay. If mangoes were rapidly cooled immediately after the hot-water immersion, mortality of immature fruit flies inside the mangoes might be reduced compared with mangoes immersed in hot water and that were not rapidly cooled. Quarantine security might be jeopardized. Hallman (in press) found that, when *A. suspensa* infesting carambolas were subjected to vapor heat (saturated hot air) at 46–46.3°C for 54 min, no larvae of an estimated 45,856 survived the treatment when the carambolas were allowed to cool in relatively still air at approximately 23°C. However, 3 of 45,856 survived when the carambolas were placed in 12.8–13°C water immediately after vapor heat treatment.

The primary objective of our research was to determine the effect to *A. suspensa* larval mortality of hydrocooling (cooling in water) infested mangoes that had previously been immersed in water at 46.1–46.7°C. One other objective was to observe the effect of hydrocooling on heat retention in mangoes.

Materials and Methods

Cooling and Larval Mortality. 'Francis' mangoes (weighing 363–461 g) from Haiti were placed in an outdoor cage (4 by 3 by 2.5 m) containing 100,000 to 300,000 *A. suspensa* adults. *A. suspensa* had been reared for approximately 180 generations on an agar diet (with wheat germ substituted for defatted wheat germ) and for approximately 200 generations on a diet based on corn cob (Burditt et al. 1975). Five to eight times more flies reared on the agar diet than the diet based on corn cob were placed in the infestation cage at any given time. Flies were replenished weekly. The mangoes remained in the fly cage for 3–5 d and then were held at 23 ± 0.5°C for 3–7 d to allow larvae to develop to the third instar; third instars bore deeper into the mango (where temperatures are lowest during hot-water immersion) than first and second instars.

The mangoes were randomly separated into five groups. Four groups were immersed in water at 46.1–46.7°C for 25, 30, 35, or 40 min as described by Sharp et al. (1988). The fifth group was untreated and was used to estimate the total number of larvae in the treated groups. Only mangoes without breaks in the skin and without soft rot were

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Table 1. Probit analyses of *A. suspensa* larval mortality in mangoes immersed in 46.1–46.7°C water for 25–40 min and then immediately cooled in air or water

Replicate	Probit 9, min	95% FL, min	Slope ± SE
Cooled in 23 ± 0.5°C air			
1	52.2	44.6–69.9	9.31 ± 1.47
2	52.1	43.5–78.3	9.46 ± 0.85
3	52.0	45.2–65.9	7.64 ± 1.04
4	59.4	37.1–>1,000	10.8 ± 4.06
$\bar{x}$[a]	53.9	—	—
Cooled in 21 ± 0.5°C water			
1	74.0	39.4–>1,000	7.88 ± 3.50
2	60.4	41.7–>1,000	7.57 ± 1.54
3	71.2	41.7–>1,000	6.68 ± 2.21
4	97.3	37.0–>1,000	3.59 ± 2.84
$\bar{x}$[a]	75.7	—	—
Cooled in 27 ± 0.5°C water			
1	120.9	40.4–>1,000	4.23 ± 3.20
2	59.1	41.8–>1,000	7.98 ± 1.52
3	58.9	45.0–136	8.74 ± 1.08
4	80.6	42.2–>1,000	5.69 ± 2.19
$\bar{x}$[a]	79.9	—	—

[a] No significant differences between means of cooling methods ($F = 2.51$; df = 2, 6; $P = 0.162$).

used. Mangoes with anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.), were used if the disease did not cover >20% of the surface area.

Water temperatures in the immersion tanks were monitored using mercury thermometers calibrated at 46.1°C with a certified precision quality thermometer (Taylor Scientific Instruments, Arden, N.C.). Immediately after each hot-water immersion, one-third each of the mangoes for each time interval were placed in a room (negligible air movement) with an ambient temperature of 23 ± 0.5°C or in 175 liters of circulating water at 21 ± 0.5 or 27 ± 0.5°C. Cooling tanks were the same type as those used for the hot-water treatment. Mangoes were placed in holding cages over vermiculite (size four) in a room at approximately 25°C to collect emerging larvae (Sharp et al. 1988). All emerging larvae were considered to be survivors. The data (four replicates) were subjected to probit analysis and analysis of variance (SAS Institute 1985, 57–82; 1988, 149–170). Test of parallelism and estimation of Probit 9 (99.9968%) mortality were done as described by Finney (1971, 107).

After Probit 9 estimates were made using results of the previous experiment, mangoes were infested as before and immersed in 46.1–46.7°C water for the mean Probit 9 estimated time for air-cooled mangoes (54 min). Ten percent of the mangoes were not heated and were used to estimate *A. suspensa* infestation rates. Half of the mangoes immersed in hot water were placed in circulating water at 21 ± 0.5° C for 1 h immediately after removal from hot-water immersion; the other half were allowed to cool in relatively still air at 23 ± 0.5°C. Larvae emerging from mangoes subjected to both cooling treatments and the unheated mangoes were collected as before.

Table 2. Sample size and tests of parallelism for probit lines (four replicates) of *A. suspensa* larval mortality in mangoes immersed in 46.1–46.7°C water for 25–40 min and then immediately cooled in water (21 or 27 ± 0.5°C) or air (23 ± 0.5°C)

Replicate	No. mangoes/treatment	Estimated no. larvae/mango	Test of parallelism	
			F statistic[a]	F probability
1	13	16.4	0.572	0.593
2	27	54.8	0.521	0.619
3	11	73.2	0.611	0.573
4	46	3.3	4.382	0.067

[a] df = 2, 6.

Heat Retention. Internal mango temperatures were monitored with data loggers (model 2200B or 2285B; John Fluke Manufacturing Company, Everett, Wash.) calibrated at 46.1°C. An insertion hole perpendicular to the mango surface was made with a needle (1 mm diameter). Copper–constantan thermocouples (no. 36 gauge) were inserted through the mango pulp to the seed (depth of 2.1–2.7 mm). The hole was sealed with approximately 0.15 ml hot melt glue (Thermogrip; Emhart Consumer Group, Reading, Pa.). Application of the adhesive did not affect the thermocouple temperatures after the glue had solidified (1–2 min). Mangoes were immersed in 46.1–46.7°C water for 54 min and then cooled in 23 ± 0.5°C still air, or circulating water at 21 ± 0.5 or 27 ± 0.5°C to determine the effect of the three cooling treatments on heat retention.

Results and Discussion

Cooling and Larval Mortality. Results of probit analyses are presented in Table 1. Number of mangoes per treatment and estimated number of larvae per mango are given in Table 2. Tests of parallelism indicated that mortality lines for larvae of *A. suspensa* resulting from the three cooling methods were parallel in all four replicates (Table 2). Therefore, results of analysis of variance should be independent of probit value. There were no significant differences among cooling methods for estimates of Probit 9 (Table 1). However, Probit 9 estimates for larvae in mangoes cooled in 23 ± 0.5°C air were consistently less than Probit 9 estimates for larvae in hydrocooled mangoes. Therefore, regardless of statistical insignificance, the probability that hydrocooling reduced mortality of *A. suspensa* larvae infesting mangoes immersed in 46.1–46.7°C water cannot be easily dismissed, considering the possible consequences of a failure of quarantine security.

A mean of 14.3 larvae per mango was estimated for the mangoes immersed in 46.1–46.7°C water for 54 min. A total of 17,589 larvae was estimated to be present in both cooling treatments. One larva

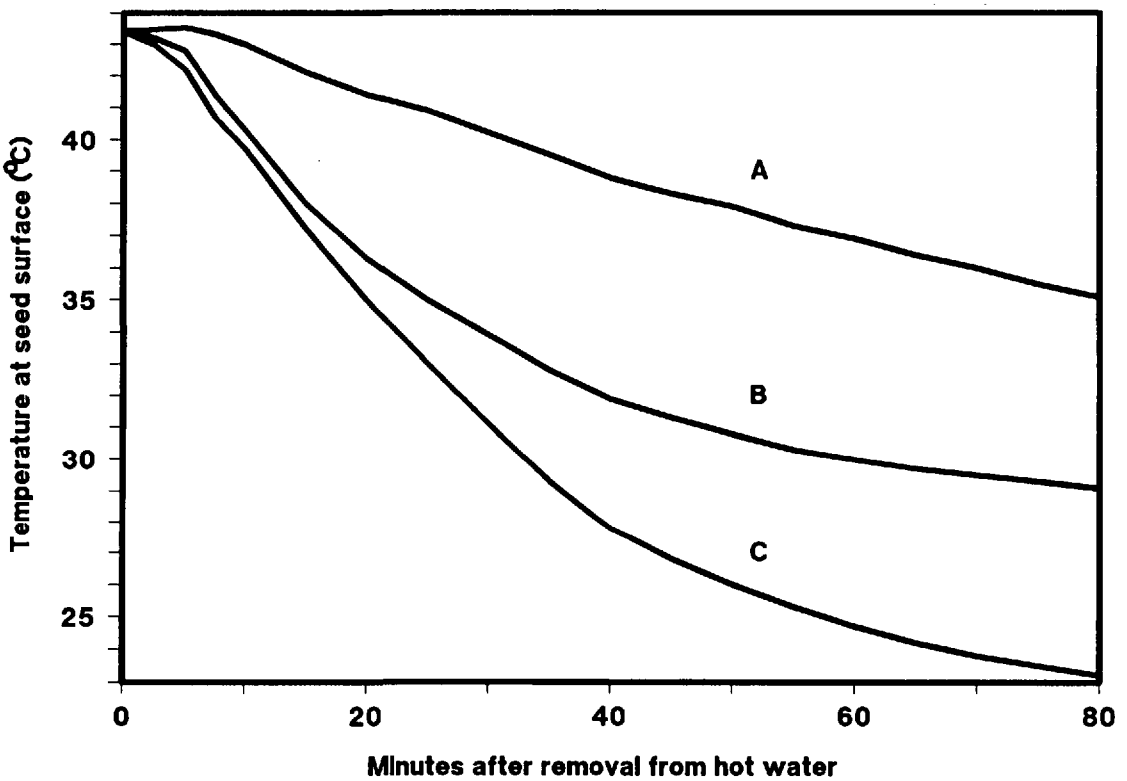

Fig. 1. Temperatures at the seed surfaces of mangoes immersed in 46.1–46.7°C water for 54 min and then cooled in 23 ± 0.5°C still air (A), or circulating water at 27 ± 0.5°C (B) or 21 ± 0.5°C (C).

survived hot-water immersion followed by immediate hydrocooling at 21 ± 0.5°C; no larvae survived in the hot-water-immersed mangoes that were cooled in relatively still air at 23 ± 0.5°C. This result provides further evidence that rapid cooling immediately after hot-water immersion may lower mortality of *A. suspensa* larvae infesting mangoes.

Heat Retention. Temperature curves for mangoes immersed in 46.1–46.7°C water for 54 min and immediately placed in relatively still ambient air at 23 ± 0.5°C or circulating water at 21 ± 0.5 or 27 ± 0.5°C are presented in Fig. 1. During the first 20 min of cooling, temperatures at the seed surface of the air-cooled mangoes fell a mean of 1.5°C, whereas temperatures at the seed surface of the mangoes placed in 21 ± 0.5 and 27 ± 0.5°C water fell 8.3 and 7.0°C, respectively. Relatively small differences in temperature can result in large differences in mortality. For example, Sharp & Chew (1987) found that in water at 40.56°C, an estimated 77.8 min were necessary to achieve 99% mortality of third-instar *A. suspensa*, whereas at 41.94°C (an increase of only 1.38°C), 43.7 min were estimated to be necessary to kill 99% of the third instars.

The effect of rapidly cooling heat-treated mangoes on the efficacy of the treatment should be considered when developing quarantine protocols. Temperature manipulation of the commodity after a heat treatment should be considered part of the quarantine treatment, such as was done by Armstrong et al. (1989). If, for concerns about market quality, mangoes needed to be rapidly cooled following heat treatment, then a longer treatment may need to be applied compared with mangoes allowed to air cool at ambient conditions. A better solution, resulting in less expenditure of energy, might be to delay cooling to allow residual heat to finish the treatment. Further experimentation is required to determine the quantity of delay necessary.

Sharp (1988) determined that *A. obliqua* was probably less susceptible to heat than *A. suspensa*. The Probit 9 estimate for *A. obliqua* infesting 'Francis' mangoes (up to 525 g) was 32% higher than the estimate for *A. suspensa*. Estimated 95% FL did not overlap greatly. Therefore, although the current quarantine treatment times for 'Francis' mangoes from the Caribbean (Anonymous 1990) are longer than the 54-min immersion time used in our research, if immediate hydrocooling were to reduce the mortality of *A. obliqua*, individuals of that species might survive the hot-water-quarantine treatment. The effect to the mortality of *A. obliqua* of rapid cooling of mangoes should be investigated if rapid cooling is allowed. Also, the effect of rapidly cooling other commodities infest-

ed with quarantine pests on mortality of the pest following heat treatment should be examined.

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