MORTALITY OF *ANASTREPHA SUSPENSA* (DIPTERA: TEPHRITIDAE) IN CARAMBOLAS TREATED WITH COLD WATER PRECOOLING AND COLD STORAGE

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ABSTRACT

The Caribbean fruit fly, *Anastrepha suspensa* (Loew), is a pest of quarantine significance of carambolas. The fruits are subjected to cold storage quarantine treatment when shipped to areas outside of the known range and where the fly could survive. In this study, rapid cooling in cold water increased mortality of Caribbean fruit fly larvae in carambolas over passive air cooling. Air-cooled carambolas required more than 24 h to cool to the treatment temperature of 1.1°C, while water-cooled fruits required only about 45 min. After 1 day, *Anastrepha suspensa* larvae had greater than 65% mortality in water-cooled carambolas, while mortality of larvae in air-cooled fruits was only 20%. Mortality of larvae in water-cooled fruits was 98% at 4 days, and 100% (1,900 larvae treated) after 9 days. Twenty six larvae were recovered from air-cooled fruits after 4 days (1,900 larvae treated), and one larva after 11 days of treatment. Larval mortality from cold-water-treated fruit reached probit 9 in 8 days, about 2/3 the time (13 days) required for the same level of mortality of larvae in air-cooled fruits. This difference in mortality is probably due to the rapidity of the cooling. It may be possible to use this modification to shorten the current cold treatment of 12 days at 1.1°C for Florida carambolas.

Key Words: Caribbean fruit fly, *Anastrepha suspensa*, commodity treatment, cold storage

RESUMEN

La mosca del Caribe, *Anastrepha suspensa*(Loew), es una plaga de la carambola, *Averrhoa carambola* L., con importancia cuarentenaria. Las carambolas de la Florida son sometidas a tratamiento cuarentenario de almacenaje en frío antes de ser enviadas a lugares donde esa mosca no existe pero tendría posibilidades de sobrevivir. En este estudio, el enfriamiento rápido en agua incrementó la mortalidad de las larvas de la mosca del Caribe en comparación con el enfriamiento pasivo mediante aire. La carambolas tratadas con aire frío requirieron un mínimo de 24 h para alcanzar la temperatura de tratamiento de 1.1°C, mientras que las enfriadas con agua requirieron solamente cerca de 45 minutos. Después de un día, las larvas de *Anastrepha suspensa* Loew tuvieron más del 65% de mortalidad en las carambolas tratadas con agua fría, mientras que las larvas de frutas tratadas con aire frío tuvieron solamente 20% de mortalidad. Las larvas de las frutas enfriadas con agua tuvieron un 98% de mortalidad a los 4 días y ninguna larva viva (de 1900 tratadas) fue recuperada 9 días después del tratamiento. Veintiseis larvas fueron recuperadas de frutas tratadas con aire frío después de 11 días de tratamiento. La mortalidad de las larvas en las frutas tratadas con agua fría llegó al Probit 9 en 8 días, cerca de 2/3 del tiempo (13 días) requerido por el mismo nivel de mortalidad de las larvas en las frutas tratadas con aire frío. Esta diferencia de mortalidad es probablemente debida a la rapidez del enfriamiento. El tratamiento de agua fría pudiera ser usado para acortar el tiempo de 12 días a 1.1°C actualmente usado para las carambolas de la Florida.

The carambola, *Averrhoa carambola* L. (Oxalidaceae), is grown throughout the tropics for its oblong, finned fruits. In south Florida the carambola is grown on 215 hectares and the acreage is increasing (Florida Agricultural Statistics Service 1996). The carambola in Florida is a poor host for the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Swanson & Baranowski 1972). A cold quarantine treatment was developed to market expanded production of carambolas (Gould & Sharp 1990, Gould 1996). Other producers of carambolas have used similar treatments (Armstrong et al. 1995). The present treatment takes 12 d at 1.1°C to produce probit 9 mortality (99.9968% mortality), a standard often used by regulatory agencies in assessing risk of introduction of exotic pests (Shannon 1994). This requires considerable lead time for marketing and shipping fruits, and the cost of keeping the fruits refrigerated at the proper temperature for 12 d is significant.

Many commodities are cooled as soon as they reach the packing house (precooling) to preserve the initial market quality of the product (Hardenburg et al. 1986). This involves rapidly cooling the produce with water, ice, or cold air. In this study we examined the effect of rapidly cooling carambolas with water at 1.0 ± .5°C on the mortality of Caribbean fruit fly larvae infesting carambolas.

MATERIALS AND METHODS

Carambolas (mean weight 175 ± 23.9 g, n = 100, 16 count) were obtained from Brooks Tropicals, Homestead, Florida. The carambolas did not have a detectable infestation in the field so they were exposed to approximately 100,000 Caribbean fruit flies in a cage for two days. The infested fruits were held at ambient conditions (26 ± 3°C) until large 3rd instar larvae developed (6-7 days). This was verified by cutting samples of five fruits.

The carambolas were then divided randomly into two treatment groups of 120 fruits and a control group of 80 fruits. The control was subdivided into 8 groups of 10 fruits. The control was held without any cold treatment and the larvae emerging were used to estimate the population present in the treated fruits. One treatment was not precooled and consisted of carambolas in commercial 16-count cardboard boxes placed directly into a walk-in cooler at 1.1 ± .75°C.

For the second treatment, fruits were precooled with cold water. Carambolas were placed in nylon mesh bags (15 × 28 cm, 35 liters) and immersed in 0.3 ± 0.1°C ice water until the fruit core temperatures approximated the water temperature (37-42 min.). The ice water was held in a 209 liter tank with a water circulating pump. Water temperature was monitored at 5 min intervals with a national standards traceable thermometer, and ice was added as necessary to maintain the temperature of the water. Thermocouples (30-gauge type t copper-constantin) were placed at the centers of three fruits in each treatment. Temperatures were monitored at 5-min intervals. Fruits were removed from the water when all of the monitored center temperatures reached 1.1°C and were placed in 16-count commercial boxes in a walk-in cooler with the other fruit from treatment No.1 (1.1 ± .75°C). At one-day intervals, 10 carambolas were removed from each treatment up to 12 days. Both treated and control fruits were held over sand for larval emergence. This experiment was replicated five times at one week intervals.

Statistical analysis was performed using probit analysis (SAS Institute 1988) and also by fitting linear and non-linear models using TableCurve 2D (Jandel Scientific 1994).

RESULTS AND DISCUSSION

The passively air-cooled carambolas in this experiment required at least 24 h to cool down to the treatment temperature (at 24 h the temperature was 2.15 ± .26°C). Water-cooled fruits cooled down in 40 (0.97 ± .35°C) to 45 min. (0.93 ± .27°C). Gould & Sharp (1990) found similar cooling curves, but did not investigate mortality from ice-water cooling.

Infestations in control fruits ranged from 134 to 753 per 80 carambolas, with a mean of 387 and std. dev. of 192. This is an average of 4.8 larvae per fruit with std. dev. of 2.4 larvae per fruit.

The mortality of insects from the two treatments was dramatically different. After one day, larvae in hydro-cooled fruits had greater than 65% mortality, while larvae in air-cooled fruits had about 20% mortality (Fig. 1A). Larvae in water-cooled fruits had 98% mortality at four days, and no larvae (from approximately 1900 larvae treated) were recovered more than 9 days after treatment. Twenty six larvae were recovered from air-cooled fruits (from approximately 1900 treated) after 8 days of treatment, and one larva was recovered after 11 days of treatment. These differences are lessened when the data are shifted one day to take into account the time it takes air-cooled fruits to cool down to treatment temperature (Fig. 1B).

The probit transformation gave the best 'fit' for the data of equations tested (using the f statistic and Tablecurve which fits 3000+ equations). Predictions for 75, 90, 99 and 99.9968% mortality are given in Table 1. Both the predictions for probit 9 for larvae in air-cooled and the shifted air-cooled fruits are close to that found by Gould & Sharp (1990). The prediction for larvae in water-cooled fruits differs greatly from predictions of mortality for larvae of either of the air-cooled data sets and the 95% fiducial limits do not overlap indicating the differences are significant. Probit 9 mortality in cold-water treated fruits was reached in just over half the time (59.8%) in air-cooled fruits. This difference in mortality is probably due to the rapidity of the cooling rather than water immersion. Taschenberg et al. (1974) found that Caribbean fruit fly larvae could survive 24 h with low mortality in water, so the water itself probably did not have a major effect on the mortality of the larvae.

Hallman (1994) found that rearing temperature did not significantly effect the mortality of Caribbean fruit fly larvae treated at 1°C. The coldest rearing temperatures used in that study, 20°C, may not have been cold enough to bring about a physiological acclimation response in the insect. Other studies have shown that in some species of flies, adults can acclimate to cold temperatures (Meats 1976, Czajka & Lee 1990, Chen & Walker 1994).

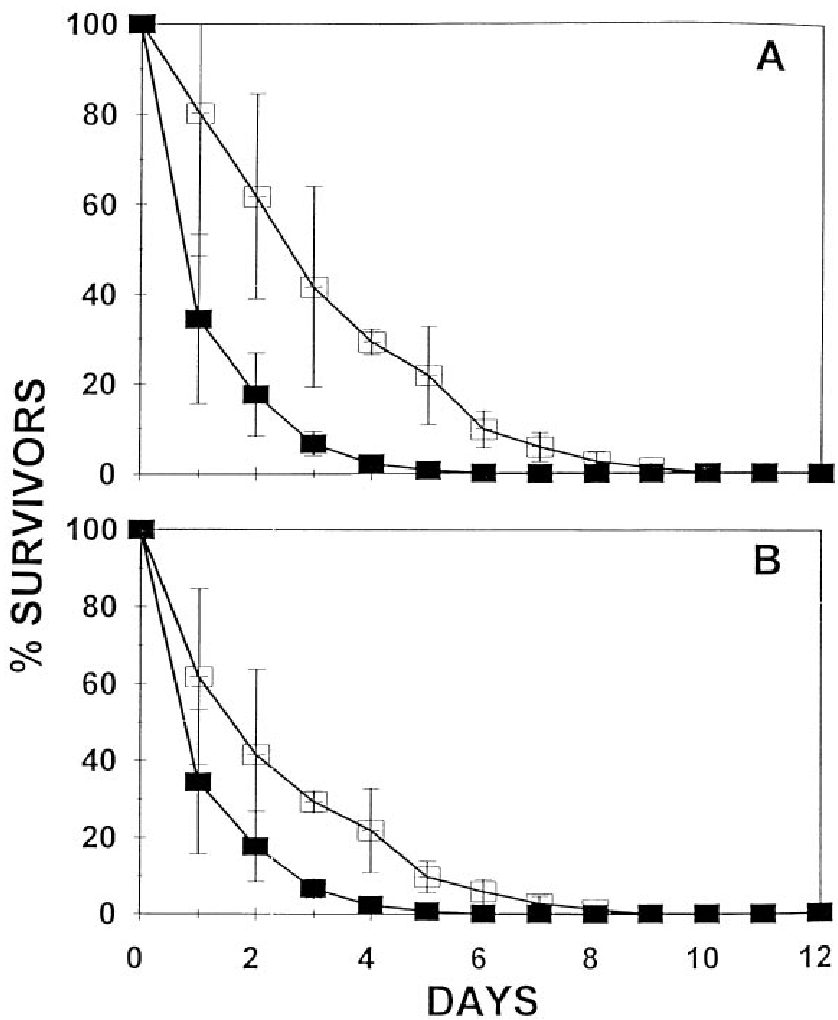

Fig. 1. Survivorship of (A) Caribbean fruit fly larvae in carambolas treated with cold water (solid square) and cold air (open square). Survivorship of (B) Caribbean fruit fly larvae in carambolas treated with cold water (solid square) and cold air (open square) adjusted by subtracting one day from cold-air treatment time to account for delayed cooling time.

The mortality of larvae over the longer period of time is presumed to be due to factors other than cold shock. Lee (1991) referred to this type of mortality as 'indirect chilling injury' with the mechanism of death presumably due to depletion of cellular resources. Lee (1991) termed the short term type of mortality as direct chilling injury or cold shock. Postulated mechanisms for cold shock include breakdown of the lipid portions of the cell wall in the temperature range of 1-2°C which allows the cellular contents to leak (Lee & Chapman 1987).

TABLE 1. MORTALITY ESTIMATES FROM COLD TREATMENTS.

Treatment	Mortality	Estimate (Days)	95% Fiducial Limits	
			Lower	Upper
Water-Cooled	75%	1.65	1.11	2.06
	90%	2.80	2.38	3.39
	99%	4.78	4.02	6.21
	99.9968%	7.94	6.44	10.95
Air-Cooled	75%	4.48	4.17	4.80
	90%	6.08	5.69	6.56
	99%	8.84	8.18	9.71
	99.9968%	13.27	12.09	14.82
Air-Cooled Shifted 1 d	75%	3.35	3.13	3.56
	90%	5.14	4.88	5.43
	99%	8.21	7.73	8.80
	99.9968%	13.14	12.22	14.28

The more rapid mortality of larvae in water-cooled fruits was probably from cold shock including the lack of time for the larvae to acclimate to the cold temperatures.

Whatever the mechanism of death, this study has shown that rapid cooling brings about rapid mortality. It may be possible to incorporate this into the current cold treatment of 12 days at 1.1°C for Florida carambolas and reduce treatment time substantially. Reductions in the treatment may make the treatment less costly and also less damaging to the fruits.

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