June, 1996

MORTALITY OF THIRD INSTAR CARIBBEAN FRUIT FLY (DIPTERA: TEPHRITIDAE) REARED IN DIET OR GRAPEFRUITS AND IMMERSED IN HEATED WATER OR GRAPEFRUIT JUICE

GUY J. HALLMAN Subtropical Agricultural Research Laboratory, USDA-ARS, 2301 S. International Blvd., Weslaco, TX 78596

Abstract

Tephritid fruit fly larval mortality due to heat has been investigated by immersing immature fruit flies into heated water. It was postulated that this information could be used to model the relationship between heat and fruit fly kill in fruits subjected to heat quarantine treatments, such as hot water immersion, vapor heat, and forced hot air. Third instar Caribbean fruit flies, *Anastrepha suspensa* (Loew), were reared on a semi-artificial diet or grapefruits and immersed in: a) tap water, b) grapefruit juice, or c) tapwater with the same pH as grapefruit juice (using citric acid), all at $43.0\pm0.05^{\circ}\text{C}$. LT_{ss} estimates were 20-31% lower for puparia and adults of third instars immersed in grapefruit juice than those immersed in heated water, with or without acid. Mortality of third instars immersed in either heated grapefruit juice or heated water was not affected by either diet.

Key Words: Quarantine treatment, hot-water immersion, modeling, Anastrepha suspensa.

RESUMEN

Ha sido investigada la mortalidad de larvas de moscas tefrítidas de la fruta mediante su inmersión en agua caliente. Se ha postulado que esta información podría ser usada para modelar la relación entre el calor y la muerte de las larvas en frutas sometidas a tratamientos cuarentenarios de calor tales como inmersión en agua caliente, calentamiento con vapor, y aire caliente forzado. Moscas fruteras del Caribe, *Anastrepha suspensa* (Loew), del tercer estadio fueron criadas en una dieta semiartificial o en toronjas e inmersas en: a) agua, b) jugo de toronja, o c) agua con el mismo pH que el jugo de toronja (usando ácido cítrico), todos a $43.0\pm0.05^{\circ}$ C. Los tiempos letales medios estimados fueron 20-30% más bajos para los puparios y adultos obtenidos de los terceros estadios sumergidos en jugo de toronja que en los inmersos en agua caliente, con o sin ácido. La mortalidad de los terceros estadios sumergidos en jugo de toronja o agua calente no fue afectada por ninguna dieta.

More research is conducted on heat quarantine treatments (hot water immersion, vapor heat, and forced hot air) by the U.S. Department of Agriculture, Agricultural Research Service, than any other type of disinfestation treatment for tephritid fruit flies (Anonymous 1992). Hot water immersion is used to disinfest mangoes shipped to the United States (U.S.) of various fruit fly species (Sharp 1994). Forced hot air is used as a fruit fly quarantine treatment for papayas grown in Hawaii and shipped to the continental U.S., and heated air treatments are preferred quarantine procedures by

168

Japan (Hallman & Armstrong 1994). Research on the mortality rate of fruit fly third instars by immersion in hot tap water has been carried out with the goal of using this information to model quarantine treatments of fruits infested with tephritids (Jang 1986, Sharp & Chew 1987, Rodriguez et al. 1989). However, the mortality response of insects immersed in hot water must be very similar to their response inside fruits for the data to be valid for modeling quarantine treatments. There are many differences between immersion of fruit fly third instars in hot water and heating of third instars inside of fruits exposed to a heat disinfestation treatment. For example, larvae used in mortality studies are usually from a colony which may be genetically different from feral flies (Chambers 1977). Fiducial limits (95%) for probit 9 estimates of time necessary to kill feral versus colony-reared West Indian fruit fly, Anastrepha obliqua (Macquart), in mangoes (mean weight = 625 g) immersed in 46.1°C water overlapped little, indicating possible differences in heat susceptibility (Sharp 1988). Larvae reared to third instar and removed from diet undergo a behavioral change as they cease feeding and seek a pupation site. Concurrently, their mortality response to heat may change. Larvae heated inside of fruits may not yet be physiologically ready to emerge from the fruit. Host may affect heat-induced mortality; larvae used in hot water immersion studies were reared on semi-artificial diets. Larvae immersed in a liquid medium are exposed to the turbulence and raised temperature immediately, while larvae in a fruit are gradually exposed to increased temperature.

Specific rearing conditions could influence mortality. Hallman (1994) found a positive relationship between rearing temperature and resistance of third instar Caribbean fruit fly, *Anastrepha suspensa* (Loew), to hot water immersion. Fruit flies used in laboratory experiments are usually reared at a high population density, whereas population densities in the field are relatively low.

Larvae heated inside of fruits are exposed to fruit pulp substances, some created because of the fruit fly infestation and waste products of the developing larvae in a semi-solid matrix, while those heated in water are in a simple liquid medium devoid of other substances. Post-treatment handling could conceivably affect survival of larvae. Larvae within a fruit would still have to complete development and emerge from the fruit, while those dipped in hot water are taken out and placed in a pupation medium, thus likely enhancing the latter's probability of survival. Therefore, there are many opportunities for the mortality response of third instars in each system to vary. The significance of each of these differences must be measured and appropriate alterations in the model be made before models can be used to accurately predict fruit fly mortality in fruits subjected to heat disinfestation treatments.

The objective of this research was to compare the heat mortality response of third instar Caribbean fruit fly reared on a semi-artificial diet or grapefruits and immersed in tap water, grapefruit juice, or tap water with the same pH as grapefruit juice at 43°C.

MATERIALS AND METHODS

The Caribbean fruit flies used in this research were from a colony reared on a semiartificial diet since 1971 at the USDA Subtropical Horticulture Research Station in Miami, Florida (Hennessey 1994). Third instars were placed in a stainless-steel wire mesh strainer and rinsed with water to remove the diet. Third instars reared on grapefruits were obtained by exposing 'Marsh' white grapefruits to oviposition in a fly cage for 4 days and then placing the grapefruits on racks where third instars would emerge and drop into a bin containing sand beneath the rack (Hallman 1994). Grapefruits were cut open once larvae began emerging, and larvae were removed directly from grapefruit pulp. Depending on the number of larvae available on any day, 25-200 larvae were placed in round stainless steel-mesh (0.5 mm between threads) tea infusers (40-mm diam). A set of five infusers each was immersed in: a) tap water (pH 7.1-9.3), b) grapefruit juice, or c) tapwater with the same pH (3.2-3.7) as grapefruit juice (using citric acid), in electrically-heated circulating water baths (11-liter capacity, Gaffney Engineering Co., Gainesville, FL) at 43.0 ± 0.05 °C. One infuser was removed after 6, 12, 18, 24, and 30 min. Immediately upon removal from the heated liquids, each infuser was cooled in a separate container of the same liquid at 24 ± 1 °C for 1 min. A sixth infuser with larvae was immersed in tap water, grapefruit juice, or acidic water at 24 ± 1 °C for 30 min as a control. The larvae were removed from the infusers and placed in 70 ml of moist No. 4 vermiculite in 0.5-liter plastic containers with screen lids.

The numbers of larvae that formed puparia and emerged as adults with functional wings and legs were counted. There were ten replicates. Numbers of puparia and adults were corrected for natural mortality (Abbott 1925) and subjected to probit analysis (LeOra Software 1987). Additionally, percentage adult mortality at 24 min immersion in $43.0\pm0.05^{\circ}$ C was subjected to analysis of variance of mortality among the treatments. Percentage adult emergence from the controls was examined with analysis of variance to determine if the immersion medium itself (without heat) caused differential mortality (SAS Institute 1988). In both of these analyses of variance, diet and heating medium were considered fixed, independent factors. Percentages were transformed by the arcsine transformation prior to analysis of variance. Differences between means were tested for statistical significance (95% confidence level) with the Ryan-Einot-Gabriel-Welsh multiple range test.

RESULTS

Probit analysis of development of Caribbean fruit fly puparia and adults heated as third instars is presented in Table 1. Fiducial limits (95% confidence level) for the immersion times estimated to kill 95% of the population (LT_{ss}) did not overlap for numbers of abnormal-looking puparia reared on agar-based diet or grapefruits and heated as third instars in grapefruit juice versus water or water with the same pH as grapefruit juice. The LT_{ss} for puparia immersed as third instars in grapefruit juice was lower than that for puparia immersed as third instars in water or water with the same pH as grapefruit juice. For adults, fiducial limits (95% confidence level) for LT_{ss} did not overlap for flies reared in grapefruits, although numerically the LT_{ss} for adults immersed as third instars in grapefruit juice was lower than that for puparia immersed as third instars in water or water with the same pH as grapefruit juice in both rearing media.

Highly significant differences occurred among heating media for adult mortality after 24 min at 43.0 ± 0.05 °C (*F* probability = 0.0006; df = 2, 33). Mean±SEM mortalities for Caribbean fruit fly adults were 98.1±0.67, 83.9±3.9, and 82.3±4.9 for third instars immersed in grapefruit juice, water, and water with the same pH as grapefruit juice, respectively, with significant differences only between the first versus the latter two immersion media. There were no significant differences between rearing diets (*F* probability = 0.93; df = 1, 33) nor any significant diet by immersion medium interaction (*F* probability = 0.84; df = 2, 33).

There were no significant differences in percentage adult emergence among the controls for diet (*F* probability = 0.85; df = 1, 51), immersion medium (*F* probability = 0.22; df = 2, 51), or diet by medium interaction (*F* probability = 0.35; df = 2, 51).

DISCUSSION

Heating Caribbean fruit fly third instars in grapefruit juice resulted in higher levels of mortality compared with the more typical practice of heating the insects in water. This increased mortality was not due to the acidity of the juice because larvae heated

170

TABLE 1. PROBIT	ANALYSIS OF	F CARIBBEAN	FRUIT FLY	Y ABNORM	AL-LOOKING	F PUPARIA	AND
ADULT	MORTALITY	FROM THIRD	INSTARS I	REARED O	N A SEMI-AF	RTIFICIAL	DIET
OR GRA	APEFRUITS AN	ND IMMERSE	D IN THRE	E LIQUID I	MEDIA AT 43	$3.0\pm0.05^{\circ}$	

Larval Diet	Immersion Medium	Slope±SEM	$LT_{_{95}}(95\%\;FL)$
Abnormal-looking p	uparia		
Semi-artificial	Water	4.1 ± 0.85	43.2(37.2-52.9)
Semi-artificial	Grapefruit juice	4.9 ± 0.90	30.0(26.7-35.1)
Semi-artificial	Acidic water	3.8 ± 0.10	43.7(35.1-61.7)
Grapefruit	Water	4.3 ± 0.18	40.3(34.4-50.3)
Grapefruit	Grapefruit juice	4.8 ± 0.18	28.1(25.2-32.1)
Grapefruit	Acidic water	5.0 ± 0.21	37.4(35.0-61.7)
Adult mortality			
Semi-artificial	Water	3.6 ± 0.75	34.3(28.5-44.9)
Semi-artificial	Grapefruit juice	$3.9{\pm}0.77$	26.9(23.2-33.2)
Semi-artificial	Acidic water	3.4 ± 0.93	33.6(25.8-54.6)
Grapefruit	Water	4.3 ± 0.17	33.0(28.3-40.8)
Grapefruit	Grapefruit juice	4.8 ± 0.18	23.9(21.5-27.5)
Grapefruit	Acidic water	5.1 ± 0.20	30.1(27.7-33.1)

in water with the same pH as grapefruit juice, using citric acid, showed the same rate of mortality as larvae heated in water alone. Quarantine treatments affect larvae in fruits, hence, it might be expected that heating larvae in fruit juice would give a more accurate simulation of mortality inside heated fruits than heating larvae in water. Nevertheless, because of the additional and abundant dissimilarities in response between *in vivo* (fruit) and *in vitro* heat treatment of fruit fly immatures, some of which were enumerated earlier, *in vitro* studies may not be useful in achieving precise estimates of heat dose required for quarantine treatments. *In vitro* studies are useful, however, in determining relative differences among various factors affecting insect mortality.

There were no significant differences in mortality of third instars reared on grapefruit versus diet and immersed in 43.0 ± 0.05 °C media indicating that diet was an adequate substitute for grapefruit in this case.

In two of 81 controls it was noted that adult emergence exceeded formation of viable-looking puparia. Examination of the puparia revealed that normal-looking adults emerged from puparia that were considered non-viable. Normal-looking adults were also noted to emerge from abnormal puparia of some of the heated larvae. Thomas & Mangan (1995) found that normal-looking adults sometimes emerged from abnormal-looking puparia of the Mexican and West Indian fruit flies, *Anastrepha ludens* (Loew) and *A. obliqua* (Macquart), respectively, following heat treatment and cautioned against the practice of considering abnormal puparia dead when researching quarantine treatments. This study provides evidence that this same phenomenon occurs with the Caribbean fruit fly.

ACKNOWLEDGMENTS

I thank Wilhelmina Wasik, USDA-ARS, Miami, FL, for technical assistance and Daniel A. Wolfenbarger, USDA-ARS, Weslaco, TX, and Lisa G. Neven, USDA-ARS, Yakima, WA, for helpful reviews.

References Cited

- ABBOTT, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.
- ANONYMOUS. 1992. Quarantine workshop for horticultural commodities: final report. U.S. Dept. Agr., Agric. Research Service.
- CHAMBERS, D. L. 1977. Quality control in mass rearing. Annual Rev. Entomol. 22: 289-308.
- HALLMAN, G. J. 1994. Mortality of third-instar Caribbean fruit fly (Diptera: Tephritidae) reared at three temperatures and exposed to hot water immersion or cold storage. J. Econ. Entomol. 87: 405-408.
- HALLMAN, G. J., AND J. W. ARMSTRONG. 1994. Heated air treatments, pp. 149-163 in J. L. Sharp and G. J. Hallman [eds.], Quarantine Treatments for Pests of Food Plants. Westview Press, Boulder, Colorado.
- HENNESSEY, M. K. 1994. Depth of pupation of Caribbean fruit fly (Diptera: Tephritidae) in soils in the laboratory. Environ. Entomol. 23: 1119-1123.
- JANG, E. B. 1986. Kinetics of thermal death in eggs and first instars of three species of fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 79: 700-705.
- LEORA SOFTWARE. 1987. POLO-PC: a user's guide to Probit Or Logit analysis. LeOra Software, Berkeley, California.
- RODRIGUEZ, A. C., G. J. HALLMAN, W. P. GOULD, AND J. J. GAFFNEY. 1989. Modeling fruit quarantine heat treatments. Paper no. 89-6053. 1989 Summer Meeting, American Soc. Agric. Engineers, Quebec.
- SAS INSTITUTE. 1988. SAS technical report: P-179, additional SAS/STAT procedures, release 6.03. SAS Institute, Cary, NC.
- SHARP, J. L. 1988. Status of hot water immersion quarantine treatment for tephritidae in mangos. Proc. Florida State Hort. Soc. 101: 195-197.
- SHARP, J. L. 1994. Hot water immersion, pp. 133-147 in J. L. Sharp and G. J. Hallman [eds.], Quarantine Treatments for Pests of Food Plants. Westview Press, Boulder, Colorado.
- SHARP, J. L., AND V. CHEW. 1987. Time/mortality relationships for Anastrepha suspensa (Diptera: Tephritidae) eggs and larvae submerged in hot water. J. Econ. Entomol. 80: 646-649.
- THOMAS, D. B., AND R. L. MANGAN. 1995. Morbidity of the pupal stage of the Mexican and West Indian fruit flies (Diptera: Tephritidae) induced by hot-water immersion in the larval stage. Florida Entomol. 78: 235-246.