Hot Water Immersion for Surface Disinfestation of *Maconellicoccus hirsutus* (Homoptera: Pseudococcidae)

ARNOLD H. HARA AND CHRISTOPHER M. JACOBSEN

Department of Plant and Environmental Protection Sciences, University of Hawaii at Manoa, College of Tropical Agriculture and Human Resources, 461 West Lanikaula Street, Hilo, HI 96720

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ABSTRACT Mealybug *Maconellicoccus hirsutus* (Green) adults, nymphs, crawlers, and eggs were tested for their susceptibility to hot water immersion at 47, 48, and 49°C. Eggs inside ovisacs were found most tolerant with prolonged survival compared with other stages at all temperatures. Ovisacs required an average of 1.38, 1.46, and 1.62 times longer treatment duration than adults, nymphs, and crawlers, respectively, for 99.9% predicted mortality at 47, 48, and 49°C. Lethal time estimations were calculated from inverse predictions of regressions derived from logit-transformed data as well as those created using a kinetic model. LT 99.9 estimations were 47.0, 21.2, and 11.9 min at 47, 48, and 49°C, respectively, by using regressions with logit transformations. The kinetic model predictions were 43.9, 19.6, and 11.1 min at 47, 48, and 49°C, respectively. During the study no emergence from eggs inside ovisacs was found after treatments of 52, 24, and 14 min at 47, 48, and 49°C, respectively. Results from this study provide efficacious temperature–time treatments.

KEY WORDS hot water immersion, mealybug, quarantine

THE MEALYBUG *Maconellicoccus hirsutus* (Green), is a USDA–Animal Plant Health Inspection Service (APHIS) quarantine pest that poses an invasive threat to many agricultural and ornamental plants in portions of the continental United States. Concern for the United States stems from the widespread distribution of *M. hirsutus* in many tropical and subtropical regions of the world, including the Caribbean and Pacific and its recorded host range of >300 plants (USDA 1998). In June 2002, *M. hirsutus* was found in Florida and became established in Broward and Miami-Dade counties initially. As of July 2004, *M. hirsutus* has spread to nine counties and nearly 1,000,000 *Anagyrus kamali* Moursi and *Gyranusoidea indica* Shafee, Alam & Agarwal parasitoids have been released in an effort to control this pest and limit its spread (Osborne 2004). In Hawaii, where this pest has been present since 1983 (Beardsley 1985), *M. hirsutus* is known to infest tropical fruits for export such as atemoya, *Annona squamosa* L. × *A. cherimola* Mill.; rambutan, *Nepthelium lappaceum* L.; durian, *Durio zibethinus* Murr.; longan, *Dimocarpus longan* (Lour.) Steud.; and sapodilla, *Manilkara zapota* L. (Follett 1999). Up to 700 eggs are laid inside white cottony ovisacs when *M. hirsutus* is reared on pumpkins (Kairo 1998). Development from egg to adult is ≈25 d for males and 26 d for females at 24–28°C (Mani 1986) and may require up to 35 d to develop under less favorable environmental conditions (Hall 1921). Male pupae develop into winged adults and live for only a few days, whereas females remain wingless and die shortly after depositing eggs; differences between the sexes are detectable by the end of the second instar (Mani 1989).

Hot water immersion is an effective disinfestation treatment against mealybugs (Lester et al. 1995; Gould and McGuire 2000). The longtailed mealybug, *Pseudococcus longispinus* (Targioni Tozetti), on persimmons was effectively controlled with 99% mortality (LT_{99}) at 46°C for 39.7 min, which decreased to 15.1 min at 54°C; mealybugs occurring under the calyx of the persimmons required an additional 6.4 min (Lester et al. 1995). Gould and McGuire (2000) documented that hot water immersion at 49°C for 20 min was effective against the citrus mealybug, *Planococcus citri* (Risso), and *P. odermatti* Miller & Williams occurring externally on limes or under the calyx. Follett (2004) reported that *M. hirsutus* was killed using vapor heat (95% RH) at 47°C for 45 min and at 49°C for 10 min with the egg stage being the most heat tolerant at the higher temperature.

The objective of this study was to determine the tolerance of *M. hirsutus* to hot water immersion for potential use in development of a postharvest disinfestation treatment. We report here the duration of hot water immersion required to effect complete mortality of each of four different *M. hirsutus* life stages at 47, 48, and 49°C.

Materials and Methods

Insect Rearing. The laboratory colony of *M. hirsutus* used in this study was initiated from *M. hirsutus* eggs

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and crawlers obtained from infested hibiscus (*Hibiscus* spp.) plants in Honolulu, HI. The colony was initially reared on Japanese pumpkin, *Cucurbita moschata* (Duchesne), and later transferred to fresh, green beans, *Phaseolus vulgaris* (L.) 'Hawaiian Wonder'. Mass rearing was accomplished by the methods of Jacobsen and Hara (2003) in which colonies of insects were held in 360-ml uncoated paper bowls (#12FCS Tapa Hawaii, Sweetheart Paper Products Co., Chelsea, MA) with plastic lids, and fresh beans were added to the bowls every 7–14 d, depending on stage and number of *M. hirsutus* inside. Mealybugs usually moved to the fresh beans independently but were occasionally transferred by brush. A dark growth chamber (Biotronette, Lab-line Inc., Melrose Park, IL) at 22°C housed bowls containing the colonies. Even-age cohorts were established by hand-collecting ovisacs and collecting crawlers that eclosed each day. Crawlers were collected on bean pods and held until they had reached the desired stage of development for treatment. Crawlers were <6 d old (days after eclosing), nymphs 12–18 d, and adults 26–40 d for all tests.

Hot Water Immersion Unit. Hot water treatments were conducted using equipment described by Hara et al. (1993). Equipment consisted of a 106-liter stainless steel tank equipped with two isotemp immersion circulators, which maintained constant tank temperature. Water temperatures were verified throughout treatment by using a digital thermometer fitted with a small temperature probe (catalog nos. 15-078-1 and 15-176-35, Fisher, Pittsburgh, PA) that had been previously calibrated with a certified liquid in glass thermometer (±0.04°C).

Hot Water Immersion of *M. hirsutus*. Egg, crawler, nymph, and adult stages of *M. hirsutus* were exposed to different durations of temperatures of 47, 48, and 49°C in 2- or 4-min increments. All temperature and immersion time combinations were replicated at least four times and up to 11 times for each of the stages. The average number of individual insects in each replicate ranged from 260 for adults to 1,087 for crawlers. Ovisacs used for treatment varied in maturity and were treated 40–54 d after eclosion of the mother, which ensured that eggs of all ages were subjected to each treatment combination. *M. hirsutus* crawlers were treated with hot water 1.5 ± 0.8 d after eclosion, whereas nymphs and adults were treated 15.8 ± 1.1 and 30.5 ± 1.8 d after eclosion, respectively. Adult, nymph, and crawler stages were treated while infesting bean pods. Ovisacs were treated on lightly crumpled filter paper that had been placed inside the containers to encourage oviposition; number of ovisacs treated was quantified after treatment. Bean pods were not a preferred oviposition site. During immersion treatment, *M. hirsutus* life stages on bean pods or eggs on filter paper were contained inside modified 150 by 20-mm polystyrene petri dishes (catalog no. U-0613902, Cole Parmer Instrument Co., Vernon Hills, IL) sealed with masking tape (Scotch brand, 3M, St. Paul, MN). Each dish was modified by cutting a 10-cm-diameter hole on the cover and bottom of the dish, which were screened with silk organza (74-μm pore size) and sealed with hot glue. This modification prevented escape of *M. hirsutus* while allowing for an exchange of hot water into the petri dishes during treatment. In addition, a small hole (5-mm-diameter) was drilled into the top of each dish, allowing for the rapid purging of air bubbles from within the dishes during initial submersion. After all air had escaped from within the dishes, the hole was covered with masking tape for the duration of the treatment. Immediately after treatment, the modified petri dishes containing *M. hirsutus* were immersed in a cooling dip of ambient water (23°C) for 2 min. Control insects were subjected to ambient water immersion for the longest treatment duration plus 2 min. After treatment, dishes containing crawler, nymph, and adult stages were isolated individually atop inverted 250-ml beakers inside 25-cm plastic moats filled with 500 ml of soapy water (1% detergent solution). Adults within the ovisacs were evaluated for mortality, and any live adults were killed to prevent posttreatment oviposition. Ovisacs were removed from the modified petri dishes, placed on filter paper, surrounded with double-sided tape (Scotch brand, 3M), and placed inside a tightly sealed 100-mm petri dish. The two-sided tape entangled crawlers as they emerged, facilitating quantification of emergence. Mortality of eggs was determined by nonemergence of crawlers and was conducted 10–14 d after treatment. Mortality, determined by absence of movement when probed with a needle, of treated crawlers, nymphs, and adults was addressed 48 h after treatment with the aid of a dissecting microscope. Absence of movement was used for mortality determination because hot water treatment preserved the color and form of the dead.

Data Analysis. Survivorship data of eggs, crawlers, nymphs, and adults were corrected for mortality in control groups with Abbott's formula (Abbott 1925). The data were then logit transformed to allow a linear plotting of the sigmoid curve relationship (Colton 1974). Simple least squares regression analyses were performed with terms Y is ln (proportion survival/1 − proportion survival) and X is minutes. Statistically significant differences in survivorship among stages at the three temperatures were determined by multiple comparisons of slope values by using Tukey's multiple comparison procedure for slopes after analysis of covariance (ANCOVA), which concluded that all slopes were not equal ($P < 0.05$) (Zar 1999). Finally, the predicted line for each stage at each temperature was backtransformed and plotted. Lethal time estimates were calculated by inverse prediction by using the equations that resulted from regression analysis (Zar 1999). Because logit transformed data do not have the same statistical reliability over the entire scale and, in common with probits, can be less reliable at the extremes or tails (Busvine 1971), a second set of lethal time predictions was created using a kinetic model detailed by Thomas and Mangan (1997) where Y is (LOG number of treated individuals − LOG number of survivors)^k and X is time. The exponential k is a death rate function derived from the reciprocal of the slope (b) of the equation LOG (LOG number of

Table 1. Regression analyses relating time in minutes (X) to logit-transformed survivorship of *M. hirsutus* after exposure to each temperature (Y) applied to eggs, crawlers, nymphs or adults (±SE)

Temp and stage	Total *N*	Obs.	y-intercept	Slope[a]	Resid. MS error	R^2	Mean (±SE) min among replicates with no survival[b]
47°C							
Egg	45,674	108	7.28 (0.36)	−0.302a (0.01)	1.91	0.85	42.7 (3.4)
Crawler	53,718	59	6.18 (0.28)	−0.448c (0.02)	1.33	0.93	29.6 (1.0)
Nymph	33,298	73	5.82 (0.33)	−0.420bc (0.02)	1.74	0.89	30.7 (0.8)
Adult	20,068	73	4.93 (0.47)	−0.364b (0.02)	2.45	0.79	31.3 (2.2)
48°C							
Egg	30,707	88	7.04 (0.29)	−0.658a (0.02)	1.46	0.91	18.6 (0.9)
Crawler	27,143	37	6.54 (0.31)	−1.05c (0.04)	1.03	0.96	12.4 (0.7)
Nymph	15,443	41	6.48 (0.33)	−0.869b (0.03)	1.10	0.95	14.7 (0.7)
Adult	12,318	54	6.26 (0.27)	−0.815b (0.02)	1.11	0.95	15.8 (0.6)
49°C							
Egg	55,402	81	7.05 (0.36)	−1.18a (0.05)	1.80	0.89	10.2 (0.5)
Crawler	41,780	25	6.62 (0.50)	−1.83b (0.10)	1.43	0.93	6.8 (0.5)
Nymph	23,072	23	6.89 (0.3)	−1.69b (0.07)	0.93	0.97	8.0 (0.0)
Adult	7,903	30	7.48 (0.41)	−1.73b (0.08)	1.30	0.94	8.0 (0.0)

[a] Within temperatures, slopes that have different letters are significantly different by Tukey's multiple comparison procedure ($P < 0.05$).
[b] Derived from the actual data by calculating the mean treatment duration with no survivors among the replicates. Predictions derived from the regression equations are provided in Table 2.

treated individuals − LOG number of survivors) = a + b(LOG time). Thermal kinetic transformation using death rate constants was originally developed for pasteurization analysis by Alderton and Snell (1970) and others and later adapted to model mortality of heat-treated fruit flies by Jang (1986, 1991). Analyses were performed using Minitab statistical software (Minitab Inc. 1997).

Results

M. hirsutus eggs inside ovisacs were the most tolerant of heat treatment at all three tested temperatures. The relationship of treatment duration and survivorship described through linear regression indicates that eggs inside ovisacs had higher survivorship, necessitating longer immersions for complete mortality (slope was less negative) compared with the other life stages at 47 ($F = 14.9$; df = 3, 305; $P < 0.0005$), 48 ($F = 28.3$; df = 3, 212; $P < 0.0005$), and 49°C ($F = 20.1$; df = 3, 153; $P < 0.0005$; Table 1; Fig. 1). The relationship between predicted 99.9% mortality of eggs and that of other stages was similar at all three temperatures; eggs required an average of 1.38 ± 0.09, 1.46 ± 0.09, and 1.62 ± 0.03 times more hot water immersion than adults, nymphs, and crawlers, respectively, regardless of temperature. The crawler stage was the most sensitive to heat treatment. Eggs were found more prone to occasional survivors at lethal temperature × treatment combinations. Therefore, greater numbers were treated to identify efficacious treatments. More than 17,000 eggs (6–11 replicates) were treated at 47°C between 32 and 52 min, and >47,000 eggs (11 replicates) were treated at 49°C for 2–14 min.

Probit 9, the statistical standard (99.9968% mortality), or a survival of ≈32 of 1,000,000 individuals, required by the USDA–APHIS for quarantine treatments, (Robertson et al. 1994) for eggs inside ovisacs immersed in 47°C water was estimated at 58.4 or 48.5 min by the logit or kinetic models, respectively; at 49°C, probit 9 was estimated at 14.8 or 12.3 min by the logit and kinetic models, respectively (Table 2). The mean duration for which there was no survival of eggs among replicates was 42.7 and 10.2 min for 47 and 49°C, respectively (Table 1); no survivors were ever found with 52 and 14 min of immersion at 47 and 49°C, respectively (Table 2). Treatment at 49°C for 12 min caused nearly complete egg mortality (99.985%) with only one survivor of 6,722 treated eggs. Estimates of 99% mortality were similar between the logit and kinetic models, differing only at 47°C by 0.1 min. However, at the 99.9968% level of mortality, the logit model was more conservative and predicted 9.9, 4.9, and 2.5 min greater duration than the kinetic model at 47, 48, and 49°C, respectively.

Discussion

Our results suggest that all stages of *M. hirsutus* would be controlled with the 20-min 49°C hot water immersion treatment that is approved for control of fruit flies in longan and lychee (Federal Register 1997, Follett and Sanxter 2002). Slightly longer immersion durations may be needed for products that absorb large quantities of heat from the water or those in which insects can find protection from the heat, as was found in the case of mealybugs under the calyxes of limes and persimmons (Lester et al. 1995, Gould and McGuire 2000). Researchers will find the temperature × time mortality results and estimates useful for developing hot water disinfestation protocols for *M. hirsutus* on specific commodities.

Our study found that the crawler stage was the most susceptible to heat and the eggs were the most tolerant. The difference in susceptibility to heat was probably due to thermodynamics rather than inherent physiological differences between stages. The small size of crawlers likely leads to efficient heat transfer into the organism for a quick kill. The hydrophobic,

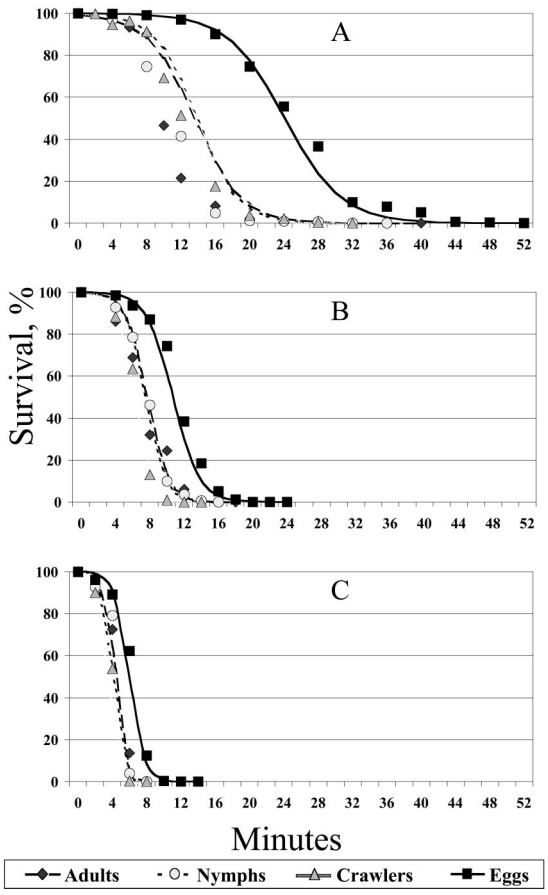

Fig. 1. Survival of *M. hirsutus* immersed in (A) 47 (B) 48, and (C) 49°C water. Statistical details for predicted lines are presented in Table 1.

waxy, flocculent material that makes up the ovisac probably insulated the egg cluster. Inside the ovisac, eggs were densely clustered, requiring heat transfer to individual eggs in the center of the egg cluster. Similarly, Hara et al. (1997) found that in hot water immersion tests against pests of red ginger flowers, surviving mealybugs "were found in copious waxy secretions." In another study, treatment of heavily infested clumps of *M. hirsutus* placed in screened vials with minimum water circulation displayed inconsistent results; *M. hirsutus* nymphs survived as high as 60°C water for three and 10 min, but 50 and 55°C water for 3 to 10 min resulted in no survivors (Parker 1996). Follett (2004) found that eggs of *M. hirsutus* were the most tolerant to vapor heat treatment at 49°C, but most susceptible at 47°C, and speculated that the waxy secretions provided protective insulation only during short duration, higher heat treatments (49°C for 3–8 min). Possibly, the dissimilar heat transfer medium, water in this study versus air in Follett (2004) caused differences in heat susceptibility of eggs clusters in ovisacs. Although the use of the logit model is appropriate for evaluating thermotolerance differences among stages, which are expressed throughout the sigmoidal survival curves, it may be less desirable for deriving predictions of efficacious treatment durations because logits are known for some unreliability at extremes of survival curves (Busvine 1971). Thomas and Mangan (1997) concluded that the kinetic based model's accuracy of predictions at the distributional tails make it preferable for estimating quarantine treatment levels. Probit 9 level mortality predictions derived from the logit model are likely overly conservative; at 48°C the estimate was 18.6% greater (4.9 min longer) than the kinetic-based prediction.

In summary, control of *M. hirsutus* with the use of hot water holds promise. Longan and lychee should effectively be disinfested with the treatment used to control fruit flies, and other host commodities that can withstand surface temperatures of 47, 48, or 49°C for 55, 23, or 13 min, respectively, are suitable candidates for hot water immersion because eggs, the most resistant stage of *M. hirsutus*, are controlled at those durations.

Table 2. Comparison of logit and kinetic models' estimations of thermal death points (95% CL) for *M. hirsutus* eggs

Temp, model	R^2	LT_{99}	$LT_{99.9}$	$LT_{99.9968}$
Logit				
47°C	0.85	39.3 (37.7–41.1)	47.0 (45.0–49.3)	58.4 (55.6–61.7)
48°C	0.91	17.7 (17.1–18.4)	21.2 (20.4–22.1)	26.4 (25.4–27.6)
49°C	0.89	9.9 (9.5–10.4)	11.9 (11.4–12.5)	14.8 (14.1–15.6)
Kinetic				
47°C	0.85	39.4 (37.8–41.1)	43.9 (42.0–46.0)	48.5 (46.4–50.9)
48°C	0.91	17.7 (17.1–18.3)	19.6 (18.9–20.3)	21.5 (20.7–22.4)
49°C	0.89	9.9 (9.5–10.4)	11.1 (10.6–11.6)	12.3 (11.8–13.0)

The actual duration (N) among all replicates with no survival was 52 (2,241), 24 (2,390), and 14 min (1,168) for 47, 48, and 49°C, respectively.

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