Thermal Death Kinetics of Red Flour Beetle (Coleoptera: Tenebrionidae)

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J. Econ. Entomol. 97(6): 1868–1873 (2004)

ABSTRACT While developing radio frequency heat treatments for dried fruits and nuts, we used a heating block system developed by Washington State University to identify the most heat-tolerant life stage of red flour beetle, *Tribolium castaneum* (Herbst), and to determine its thermal death kinetics. Using a heating rate of 15°C/min to approximate the rapid heating of radio frequency treatments, the relative heat tolerance of red flour beetle stages was found to be older larvae > pupae and adults > eggs and younger larvae. Lethal exposure times for temperatures of 48, 50, and 52°C for the most heat-tolerant larval stage were estimated using a 0.5th order kinetic model. Exposures needed for 95% mortality at 48°C were too long to be practical (67 min), but increasing treatment temperatures to 50 and 52°C resulted in more useful exposure times of 8 and 1.3 min, respectively. Red flour beetle was more sensitive to changes in treatment temperature than previously studied moth species, resulting in red flour beetle being the most heat-tolerant species at 48°C, but navel orangeworm, *Amyelois transitella* (Walker), being most heat tolerant at 50 and 52°C. Consequently, efficacious treatments for navel orangeworm at 50–52°C also would control red flour beetle.

KEY WORDS heat treatments, red flour beetle, radio frequency, dried fruits, tree nuts

CALIFORNIA PRODUCES NEARLY ALL of the walnuts, almonds, and pistachios grown in the United States, resulting in an annual production of >700,000 metric tons of commodity valued at >$1 billion (USDA 2003). A large portion of California tree nuts (>400,000 metric tons) are sold for the export market. A major problem in the storage and marketing of California tree nuts is infestation by a variety of postharvest insect pests, including field pests of possible phytosanitary importance such as navel orangeworm, *Amyelois transitella* (Walker), and codling moth, *Cydia pomonella* (L.), as well as common stored-product pests such as Indianmeal moth, *Plodia interpunctella* (Hübner), and red flour beetle, *Tribolium castaneum* (Herbst) (Simmons and Nelson 1975). Currently, the dried fruit and tree nut industry relies on fumigation with methyl bromide and phosphine (hydrogen phosphide) for postharvest insect control (Carpenter et al. 2000). Regulatory actions against methyl bromide (UNEP 1992) and hydrogen phosphide (EPA 1998), as well as insect resistance to hydrogen phosphide (Zettler et al. 1989), may make these fumigants costly or unavailable to the nut industry. In addition, as the organic industry expands, the need for nonchemical postharvest insect control methods increases. Although nonchemical treatments for postharvest dried fruits and nuts have been investigated in the past (Storey and Soderstrom 1977), few have been implemented. Recent concerns over resistance, regulatory action and the needs of the organic industry have generated a renewed interest in developing alternative treatments.

Possible nonchemical alternative methods to control postharvest insects include ionizing radiation, cold storage, controlled atmospheres, and combination treatments (Johnson et al. 2003). All have disadvantages such as substantial capital investment, extensive alteration of existing facilities, lengthy treatment times, or concerns over consumer acceptance. Heat treatments using forced hot air also have been proposed, but the lengthy exposure times (>1 h) needed to heat nuts throughout may substantially reduce product throughput or cause product damage. Industrial radio frequency systems, extensively used in the food, textile, and wood processing industries, may avoid these problems by providing more rapid product heating (10–20°C/min) and have been suggested for control of postharvest insects (Hallman and Sharp 1994, Nelson 1996, Tang et al. 2000). Recently, radio frequency treatments have been shown to kill codling moth and navel orangeworm in in-shell walnuts (Wang et al. 2001, 2002c). Knowledge of thermal death kinetics for targeted insects is essential in developing thermal treatments by using microwave or

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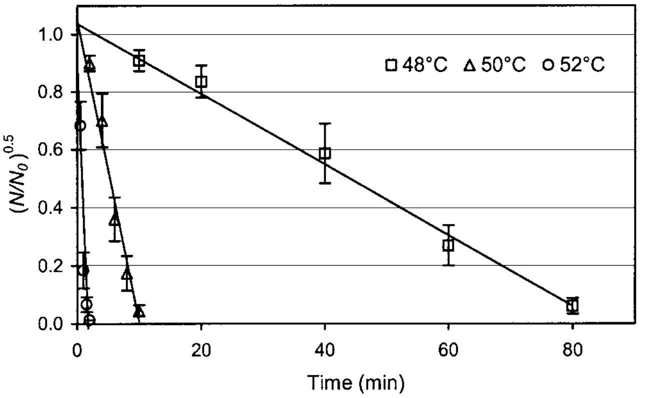

Fig. 1. Thermal mortality curves of older red flour beetle larvae at three different temperatures using an 0.5th order kinetic model.

radio frequency heating, allowing us to select potentially useful treatment schedules for further testing.

Methods used for studying thermal death kinetics of insects include directly exposing insects in a water bath for specific times, heating insects in tubes that in turn are submerged in a water bath, or heating insects in fruits (Yokoyama et al. 1991, Thomas and Mangan 1997, Waddell et al. 2000). A heating block system developed at Washington State University (WSU), Pullman, WA (Ikediala et al. 2000; Wang et al. 2002a, b) directly heats exposed insects and provides precise heating rates from 1 to 20°C/min. The heating block is particularly useful for simulating the rapid heating rates of radio frequency treatments, and data from previous heat block studies have proven useful in providing radio frequency treatment schedules (Wang et al. 2001, 2002c).

The WSU heating block system was used to determine heat tolerance of fifth instars of codling moth (Ikediala et al. 2000, Wang et al. 2002a), navel orangeworm (Wang et al. 2002b), and Indianmeal meal moth (Johnson et al. 2003). In this study, we used the WSU heating block system to identify the most heat-tolerant life stage of red flour beetle and determine its thermal death kinetics at a heating rate of 15°C/min.

Materials and Methods

Heating Block System. The WSU heating block system consisted of top and bottom aluminum blocks (each 254 by 254 by 18 mm), heating pads, an insect test chamber, and a data acquisition/control unit. Detailed descriptions of the heating system can be found in Ikediala et al. (2000) and Wang et al. (2002b). Calibrated type-T thermocouples inserted through sensor paths were used to monitor the temperatures of the top and bottom blocks. Heating rate, set-point temperature, and exposure time were computer controlled by a customized Visual Basic program and PID controllers (i/32 temperature & process controller, Omega Engineering Inc., Stamford, CT) via a solid-state relay. The heating block system used in this study was slightly different from that in earlier studies, and the maximum heating rate was 15°C/min rather than 20°C/min.

Test Insects. Red flour beetle used in the study were from a long-term (>20-yr-old) laboratory culture maintained on a mixture of rice bran, whole wheat flour, and brewers yeast, by using the methods outlined by Bry and Davis (1985). Cultures were kept in 1 liter canning jars at 25°C, 50% RH, and a photoperiod of 14:10 (L:D) h. Adults, pupae, older larvae, and younger larvae were removed from culture jars by using variously sized sieves. Eggs were obtained by placing adults overnight in processed flour that had been passed through a 100 mesh sieve. The resulting eggs were removed by passing the flour through a 60 mesh sieve. All test insects, including eggs, were transferred by means of a vacuum aspirator to plastic petri dishes just before treatment.

Experimental Protocol. A heating rate of 15°C/min was selected to simulate the rapid heating of nuts subjected to radio frequency and microwave energies (Wang et al. 2001), resulting in treatment temperatures of ≥48°C being reached in ≈2 min. To determine the most heat-tolerant life stage, eggs, young larvae (approximately second instar), old larvae (approximately sixth to eighth instar), pupae, and adults were exposed to temperatures of 48°C for 20, 30, and 40 min; 50°C for 4, 8, and 12 min; and 52°C for 0.5, 1, and 2 min. Control insects were placed in the unheated block chamber for 40 min, equivalent to the longest treatment exposure. Approximately 50 test insects of each stage were treated at each temperature–time combination, including controls. The test was replicated four times.

The stage found to be the most heat-tolerant was used in subsequent thermal death kinetic studies. Four or five exposure times (0.5–80 min) at 48, 50, and 52°C were selected to provide a wide range of mortality levels (Fig. 1). Control insects were placed in the unheated block for 100 min. Approximately 100–150 insects were treated at each temperature–time combination, including controls. The test was replicated five times, with a minimum of 600 insects treated at each temperature–time combination.

To begin each treatment, test insects were poured from the dishes onto the bottom heating block. The top block was then placed on the bottom block and the treatment program begun. At the end of each exposure, test insects were quickly (within 10 s) transferred to plastic petri dishes, by using a vacuum aspirator. Treated insects were held at 25°C, 50% RH, and a photoperiod of 14:10 (L:D) until evaluation. Larvae and adults were evaluated 24–48 h after treatment and were considered to be dead if no movement was observed. Eggs and pupae were held until hatch or adult emergence, respectively, usually 10–14 d.

Treatment Model and Statistical Analysis. Mortality was calculated as the proportion of dead insects relative to total treated insects for each treatment and life stage, corrected for control mortality using Abbott's formula (Abbott 1925). Mean values and standard deviations were obtained from all replications for each temperature–time combination. Treatment mortality for different life stages was compared for each temperature–time combination by using the SAS analysis of variance (ANOVA) procedure (SAS Institute 1989). We used an arcsine square-root transformation to normalize the data before analysis. Where ANOVA showed statistical differences ($P \leq 0.05$) between life stages, means were separated using least significant difference (LSD) (SAS Institute 1989). In developing the thermal death model for the most tolerant life stage, a value of 0.1 was used for the number of surviving insects where actual survival was 0%.

We used a fundamental kinetic model to describe the response of the most tolerant life stage to heat and allow us to estimate LT_{95} and LT_{99} values. The model is similar to that previously used for codling moth (Wang et al. 2002a), navel orangeworm (Wang et al. 2002b), and Indianmeal moth (Johnson et al. 2003) and is based on the following equation:

$$\frac{d(N/N_0)}{dt} = -k(N/N_0)^n \qquad [1]$$

where N_0 and N are the initial and surviving numbers of insects, t is the exposure time (minutes) at a fixed temperature, k is the thermal death rate constant (1/minute), and n is the kinetic order of the reaction. The integration form of equation 1 can be obtained for different reaction orders as follows:

$$\begin{aligned} \ln(N/N_0) &= -kt + c \quad (n = 1) \\ (N/N_0)^{1-n} &= -kt + c \quad (n \neq 1) \end{aligned} \qquad [2]$$

For each temperature, survival (N/N_0) was regressed against exposure time (t) according to equation 2 for the 0, 0.5, 1, 1.5, and 2 reaction orders. The most suitable reaction order was determined by comparing the coefficients of determination (r^2) for all treatment temperatures. After the reaction order was fixed, the values of k and c were derived from the regression equation and used to estimate the LT_{95} and LT_{99} for each treatment temperature.

A thermal death time (TDT) curve for the most tolerant stage was developed by plotting for each temperature the minimum exposure time required to achieve 100% mortality of test insects on a semilog scale (Ikediala et al. 2000, Wang et al. 2002b). The z value (the temperature difference for which the mortality rate is altered by a factor of 10), calculated as the negative inverse of the slope of the TDT curve, was used to derive the activation energy (E_a, J/mol) needed for thermal death of test larvae according to the following relationship (Tang et al. 2000, Wang et al. 2002b):

$$E_a = \frac{2.303RT_{\min}T_{\max}}{z} \qquad [3]$$

where R is the universal gas constant (8.314 J/mol K), and $T_{\min}$ and $T_{\max}$ are the minimum and maximum temperatures (K) of a test range, respectively. Activation energy for thermal death of test larvae also was calculated from the slope of an Arrhenius plot of $\log k$ versus the reciprocal of the absolute temperature ($1/T$) (Tang et al. 2000, Wang et al. 2002b) as follows:

$$k = k_{ref}e^{\frac{-Ea}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)} \qquad [4]$$

where T is the absolute temperature (K), and k_{ref} is the thermal death rate constant at the reference temperature T_{ref} (K).

Table 1. Mortality of different life stages of red flour beetle exposed to high temperatures

Exposure (min)	Eggs	Younger larvae	Older larvae	Pupae	Adults
48°C					
20	100.0a	96.2 ± 1.2ab	24.7 ± 2.6c	72.9 ± 19.7b	78.6 ± 5.8b
30	100.0a	99.0 ± 0.6a	30.8 ± 4.9c	99.0 ± 0.6a	86.8 ± 8.7b
40	100.0a	97.6 ± 2.4a	57.1 ± 12.0b	100.0a	96.9 ± 2.0a
50°C					
4	94.0 ± 6.0a	97.3 ± 1.1a	30.0 ± 11.9c	53.2 ± 4.2cb	62.2 ± 10.2b
8	100.0a	99.5 ± 0.5a	74.9 ± 9.0b	99.4 ± 0.5a	97.0 ± 1.3a
12	100.0a	100.0a	97.8 ± 0.9b	100.0a	100.0a
52°C					
0.5	99.2 ± 0.8a	92.3 ± 2.2a	27.5 ± 15.4b	46.1 ± 6.8b	52.3 ± 12.4b
1	98.9 ± 1.1a	96.5 ± 2.8a	78.2 ± 9.8a	73.1 ± 13.8a	81.4 ± 7.5a
2	99.2 ± 0.8a	92.9 ± 5.0a	100.0a	99.4 ± 0.6a	96.3 ± 1.3a

Values are means ± SE of four replicates. Within rows, values followed by a different letter are significantly different (LSD mean separation, $P \leq 0.05$).

Table 2. Coefficients of determination (r^2) from kinetic order (n) models for thermal mortality of older red flour beetle larvae at three temperatures

Temp (°C)	$n = 0$	$n = 0.5$	$n = 1$	$n = 1.5$	$n = 2$
48	0.967	0.992	0.880	0.658	0.550
50	0.935	0.977	0.886	0.612	0.477
52	0.791	0.898	0.966	0.654	0.530

Results and Discussion

Most Heat-Tolerant Life Stage. Percentage of mortality of different red flour beetle life stages is given in Table 1. Overall, older larvae were the most tolerant, particularly at lower treatment temperatures. Mortality levels of older larvae were significantly less than other stages at all exposures at 48°C ($F = 10.61–41.60$; df = 4, 15; $P \leq 0.001$) and 50°C ($F = 8.01–13.67$; df = 4, 15; $P \leq 0.001$). At 52°C, mortality of older larvae was significantly different from eggs and younger larvae for only the 0.5-min exposure ($F = 13.81$; df = 4, 15; $P \leq 0.0001$); no differences were noted between mortality levels for older larvae, pupae, and adults at this exposure. There were no significant difference between any of the stages at 52°C for 1- and 2-min exposures. No significant differences were seen between eggs and younger larvae at any temperature–time combination, and these two stages were usually the least heat-tolerant. Pupae and adults were significantly more tolerant than eggs and younger larvae at 48°C for 20 min and 52°C for 0.5 min. Based on these results, relative heat tolerance of red flour beetle stages was as follows: older larvae > pupae and adults > eggs and younger larvae.

Fields (1992) found very little in the literature on relative heat tolerance of *Tribolium* life stages. Oosthuizen (1935) found that pupae of the confused flour beetle, *Tribolium confusum* Duval, were most tolerant at 44 and 46°C, and adult confused flour beetles were more tolerant at 48 and 50°C. Kirkpatrick and Tilton (1972), working with an infrared heat source and very rapid heating rates of ≈60°C/min, reported adult *T. castaneum* survival as 38, 2, and 0.4% after brief exposures to 49, 57, and 66°C, respectively, but they did not look at other life stages. More recently, Mahroof et al. (2003a, b) reported variable results in studies on the development of heat treatments for buildings. Red flour beetle pupae were found to be most tolerant during heat treatments of pilot feed and flour mills (Mahroof et al. 2003a), whereas laboratory studies based on exposures to constant temperatures found that young larvae were the most tolerant, especially at temperatures of >50°C (Mahroof et al. 2003b). Differences between the studies may be due to differences in heating conditions, particularly in the variability of heating conditions found during heat treatments of whole buildings.

Mahroof et al. (2003b) reported variable heating rates of ≈1.5°C/min to 46°C and 3°C/min for 50°C. To simulate the heating rates obtained under radio frequency heat treatments, we used a more rapid heating rate of 15°C/min. Mahroof et al. (2003b) suggested various possible reasons for the increased heat tolerance found in young larvae, including the production of heat shock proteins. Mahroof et al. (2003b) also noted that respiration of young larvae has been shown to be markedly higher than other life stages (Emekci et al. 2002) and that this may be attributable to higher metabolic rates often associated with a reaction to stress and may enhance survival under unfavorable conditions. If the increased heat tolerance of young larvae noted by Mahroof et al. (2003b) is due to the production of heat shock proteins or some other metabolic process, then the more rapid heating rate used in our study may have prevented a similar acclimation. Slow heating rates have been found to increase the production of heat shock protein in other insects. Thomas and Shellie (2000), while working with heat treatments for the Mexican fruit fly, *Anastrepha ludens* (Loew), noted that a presumptive heat shock protein (32 kDa) was produced in 76% of larvae treated at a slow heating rate (0.175°C/min), whereas only 42% of larvae treated at a faster heating rate (1.4°C/min) produced the same protein.

Thermal Death of Older Larvae. Based on the results of our life stage comparisons, we selected older larvae for subsequent thermal death studies. Mortality of untreated larvae was low (0.7 ± 0.4%), suggesting that handling had little effect on test insects. Coefficients of determination (r^2) derived for each kinetic order model and treatment temperature are given in Table 2. As we found for codling moth (Wang et al. 2002a), navel orangeworm (Wang et al. 2002b), and Indianmeal moth (Johnson et al. 2003), the 0.5th order reaction model (Fig. 1) produced the most consistently high r^2 values (0.898–0.992) for all temperatures. Consequently, we chose this model for further calculations.

For 48, 50, and 52°C, the 0.5th order model parameters, estimated lethal times for 95 and 99% mortality, and observed minimum exposure for 100% mortality of

Table 3. Observed exposure (minutes) to achieve 100% mortality, model parameters, and estimated lethal times (minutes) for older red flour beetle larvae exposed to three temperatures

Temperature (°C)	N_t *	Observed min. for 100% mortality of 600 insects	Parameters (±SE) and estimated lethal times for 0.5 order kinetic model $(N/N_0)^{0.5} = -kt + c$						
			k	c	r^2	LT_{95}	95% CI	LT_{99}	95% CI
48	3,116	85	0.0122 ± 0.0006	1.0365 ± 0.0384	0.992	66.6	61.2–72.0	76.8	70.4–83.2
50	3,151	12	0.1042 ± 0.0080	1.0509 ± 0.0666	0.977	7.9	6.9–8.9	9.1	8.0–10.2
52	2,543	2	0.5182 ± 0.1006	0.9075 ± 0.1591	0.898	1.3	0.8–1.8	1.6	1.1–2.1

* N_t is total number of test insects per temperature, about 100–150 insects for each of five replicates and four to five exposures.

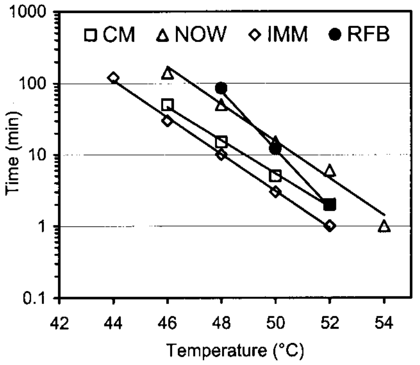

Fig. 2. Comparison of TDT curves of red flour beetle with three common postharvest pests of tree nuts. Lines represent linear regression equations where t is time (minutes) and T is temperature (°C). Older red flour beetle larvae (RFB): $\log t = 21.46 - 0.41\ T$. Fifth instars of codling moth (CM): $\log t = 12.41 - 0.23\ T$. Fifth instars of navel orangeworm (NOW): $\log t = 14.193 - 0.261\ T$. Fifth instars of Indianmeal moth (IMM): $\log t = 13.39 - 0.26\ T$. In all cases, $r^2 > 0.99$ and $N = 600$.

600 insects are given in Table 3. Although our previous life stage studies resulted in some survival of older larvae at 12-min exposure to 50°C, subsequent thermal death tests showed no survival at this treatment combination; consequently, we selected 12 min for the observed minimum exposure for 100% mortality at 50°C. Red flour beetle larvae were very tolerant of 48°C, with an estimated LT_{95} and LT_{99} value of 67 and 77 min, respectively. These exposure times are not practical for rapid treatment of large volumes of product, particularly if the product is heat-sensitive. The estimated LT_{95} and LT_{99} values for 50°C (8 and 9 min, respectively) and especially 52°C (1.3 and 1.6 min, respectively) were much more acceptable as treatment times and have been shown to have no detrimental effect on product quality (Wang et al. 2002c).

In previous studies with codling moth (Wang et al. 2002a), navel orangeworm (Wang et al. 2002b), and Indianmeal moth (Johnson et al. 2003), we compared the results derived from the 0.5th order kinetic model

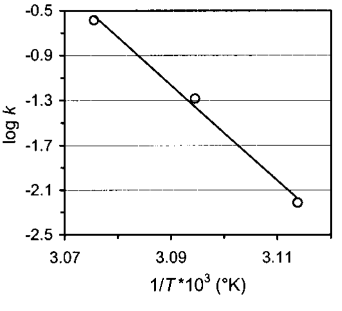

Fig. 3. Arrhenius plot for temperature effects on thermal death rates for older red flour beetle larvae. Line represents linear regression equation $\log k = 130.21 - 42.52*1000/T$ ($r^2 = 0.99$) where k is thermal death rate constant and T is temperature.

with an empirical model (Alderton and Snell 1970). The empirical method is a modification of the first order reaction model with the addition of an exponential constant to improve curve fitting. But this constant does not carry any obvious information to help interpret the thermal death kinetics of insects. In all our previous studies, the 0.5th order kinetic model was preferred because it provides precision similar to that of the empirical model, is considerably easier to calculate, and results in more conservative treatment times. Consequently, we decided not to include the empirical model in this study.

Thermal Death Time Curve and Activation Energy. The TDT curve for older red flour beetle larvae is given in Fig. 2. The curve defines minimum time–temperature combinations to achieve 100% mortality in a sample of 600 insects. The curve for red flour beetle is described by the linear regression equation $\log t = 21.46 - 0.41\ T$ ($r^2 = 0.99$) where t is time and T is temperature. The z value for older red flour beetle, derived from the negative inverse of the slope of the TDT curve, is 2.456°C, and yields a thermal death activation energy value of 814.1 kJ/mol. An alternative method to calculate activation energy from the slope of an Arrhenius plot (Fig. 3, equation 4 for rate constant, k, resulted in an estimation of 814.0 kJ/mol.

The activation energy is useful in determining the sensitivity of insects to changes in temperature and is determined by the slope of the TDT curve. Comparison of the TDT curves for the insects we have tested so far (Fig. 2) shows that the slope of the curve for red flour beetle was higher than for the three moth species. Consequently, the activation energy for thermal death of red flour beetle larvae calculated from the TDT curve is considerably greater than that found for navel orangeworm (519 kJ/mol) (Wang et al. 2002b), codling moth (472 kJ/mol) (Wang et al. 2002a), and Indianmeal moth (506.3 kJ/mol) (Johnson et al. 2003), indicating that red flour beetle is more sensitive to

Table 4. Comparison of the observed minutes of exposure for 100% mortality, and LT_{95} and LT_{99} in minutes for red flour beetle, navel orangeworm, codling moth, and Indianmeal moth

Species	Observed min for 100% mortality of 600 insects	LT_{95}	LT_{99}
48°C			
Red flour beetle	85	66.6	76.8
Navel orangeworm	50	36.0	42.3
Codling moth	15	10.6	12.4
Indianmeal moth	10	7.6	8.8
50°C			
Red flour beetle	12	7.9	9.1
Navel orangeworm	15	11.5	13.3
Codling moth	5	3.6	4.2
Indianmeal moth	3	2.2	2.5
52°C			
Red flour beetle	2	1.3	1.6
Navel orangeworm	6	3.7	4.5
Codling moth	2	1.4	1.7
Indianmeal moth	1	0.9	1.0

Data for codling moth, navel orangeworm, and Indianmeal moth are from Wang et al. (2002a, b) and Johnson et al. (2003), respectively.

temperature change. In practical terms, whereas red flour beetle is the most heat-tolerant at 48°C, with LT_{95} and LT_{99} values nearly twice those of the most tolerant moth species, at 50 and 52°C red flour beetle is less tolerant than navel orangeworm (Table 4). Because target temperatures for radio frequency heat treatments will most likely be ≥55°C (Wang et al. 2002c), we will continue to consider fifth instars of navel orangeworm larvae the most heat-tolerant species and life stage of the insect pests associated with California tree nuts. Efficacious heat treatments developed for navel orangeworm at 50–52°C also will control red flour beetle.

Acknowledgments

This research was supported by Washington State University and grants from USDA–NRI (99-35316–8099) and USDA–IFAFS (00-52103–9656) programs. We thank James Hansen (USDA–ARS, Wapato, WA) and Peter Follett (USDA–ARS, Hilo, HI) for reviewing the manuscript.

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Received 30 April 2004; accepted 17 September 2004.