Thermal Death Kinetics of Fifth-instar *Plodia interpunctella* (Lepidoptera: Pyralidae)

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J. Econ. Entomol. 96(2): 519–524 (2003)

ABSTRACT Heat treatments have been suggested as alternatives to chemical fumigants for control of postharvest insects in dried fruits and nuts. Conventional forced hot air treatments heat product too slowly to be practical, but radio frequency treatments are capable of more rapid product heating. While developing radio frequency heat treatments for dried fruits and nuts, the heat tolerance of nondiapausing and diapausing fifth-instar larvae of the Indianmeal moth, *Plodia interpunctella* (Hübner), was determined using a heating block system developed by Washington State University. Both a 0.5th order kinetic model and a classical empirical model were used to estimate lethal exposure times for temperatures of 44–52°C for nondiapausing fifth-instar larvae. We obtained 95% mortality at exposures suitable for practical radio frequency treatments (≤5 min) with temperatures of 50 and 52°C. Diapausing larvae were significantly more tolerant than nondiapausing larvae at the lowest treatment temperature and shortest exposure, but differences were not significant at more extreme temperature-time combinations. Previous studies showed that fifth-instar larvae of the navel orangeworm, *Amyelois transitella* (Walker), were more heat tolerant than either diapausing or nondiapausing Indianmeal moth larvae. Consequently, efficacious treatments for navel orangeworm would also control Indianmeal moth.

KEY WORDS Heat treatments, Indianmeal moth, radio frequency, postharvest, dried fruits, tree nuts

CALIFORNIA PRODUCES NEARLY ALL of the dried fruits and nuts in the United States, resulting in an annual production of >1.2 million metric tons of commodity valued at over $1 billion (USDA 2000). A major problem in the storage and marketing of dried fruits and nuts in California is infestation by Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Simmons and Nelson 1975). Larvae of this cosmopolitan storage pest reduce nut quality, safety, and marketability through feeding and by contaminating product with frass and webbing. 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 (USEPA 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 (Storey and Soderstrom 1977), recent concerns over resistance, regulatory action and the needs of the organic industry have generated a renewed interest in developing alternative treatments.

Several nonchemical alternative methods to control Indianmeal moth infestations have been suggested, including ionizing radiation (Johnson and Marcotte 1999), cold storage (Johnson et al. 1997), controlled atmospheres (Brandl et al. 1983), and combination treatments (Johnson et al. 1998). 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 or hot water dips have been proposed for a variety of postharvest insect pests (Hallman and Armstrong 1994, Sharp 1994, Burks et al. 2000), but the lengthy exposure times needed may reduce product throughput or cause product damage. Industrial radio frequency and microwave systems, extensively used in the food processing, 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 (Andreuccetti et al. 1994, Hallman and

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Sharp 1994, Nelson 1996, Tang et al. 2000, Wang et al. 2001). Knowledge of thermal death kinetics for targeted insects such as the Indianmeal moth is essential in developing thermal treatments using microwave or radio frequency heating.

Several methods have been reported for studying thermal death kinetics of insects. These methods include directly exposing insects in a water bath for specific times, heating insects in tubes which 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). Recently, a heating block system was developed at Washington State University (WSU), Pullman WA, to study thermal death kinetics of postharvest insects without the use of heated water (Ikediala et al. 2000, Wang et al. 2002a, b). This system directly heated exposed insects and accurately simulated different heating rates from 1 to 20°C/min, matching the heating rates of most practical heat treatments. It is particularly useful for estimating treatment temperatures and exposures for the rapid heating rates of radio frequency treatments.

The WSU heating block system was used to determine heat tolerance of codling moth (Ikediala et al. 2000, Wang et al. 2002a) and navel orangeworm (Wang et al. 2002b), using fifth-instar larvae as test insects. Preliminary data with navel orangeworm indicate that the fifth-instar is the most heat tolerant stadium (Wang et al. 2002c). In this study, we used the WSU heating block system to determine the thermal death kinetics of fifth-instar Indianmeal moth larvae at a heating rate of 18°C/min, and compare their relative heat tolerance to the previous target species. As diapausing larvae are often found to be the stadium most tolerant of postharvest treatments (Bell 1994) such as fumigation (Bell 1977a, b), cold storage (Bell 1982) and controlled atmospheres (Soderstrom et al. 1990), we also compared the relative heat tolerance of diapausing and nondiapausing Indianmeal moth larvae.

Materials and Methods

Heating Block System. The WSU heating block system consisted of top and bottom blocks, 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, and the air temperature in the chamber. Heating rate (0.1–20°C/min) and the set-point temperature were computer controlled using WorkBench PC 2.0 (Strawberry Tree Inc., Sunnyvale, CA) via a solid-state relay. The heating block unit was insulated to prevent heat loss during the tests.

Test Insects. Test insects were from an Indianmeal moth laboratory colony originally obtained from a walnut packinghouse in Modesto, CA, in November 1967 and were maintained at the USDA Horticultural Crops Research Laboratory, Fresno, CA, on a wheat bran diet (Tebbets et al. 1978). Rearing conditions for nondiapausing larvae were 27°C, 60% RH, and a photoperiod of 14:10 (L:D) h.

To produce diapausing larvae, in late October 2000 we added Indianmeal moth eggs to plastic deli cups (475 ml) containing 250 g of wheat bran diet. We provided ventilation by taping organdy cloth over a 2.2-cm hole punched into the center of each deli cup lid. We held the cups under rearing conditions for 1 wk, and then moved them to a covered, unheated storage area, free from artificial light. Approximately 5 wk later, we moved the cups to a 10°C refrigerated storage unit, held them there for 3 wk, and then shipped them to WSU for testing.

Before treatment, nondiapausing fifth-instars were removed from rearing jars and placed in plastic deli cups (475 ml) filled with diet. Deli cups containing either diapausing or nondiapausing larvae were packed in insulated shipping cartons, and shipped via overnight delivery to WSU. Data loggers (Hobo H8 temperature logger, Onset Computer, Bourne, MA) were included in each box to monitor temperatures in transit. Once at WSU, the insects were transferred to glass jars (1.65 liters) and left at room temperature for several hours before testing. We used only actively moving fifth-instar larvae in tests.

Experimental Protocol. A heating rate of 18°C/min was selected to simulate the rapid heating of fruit subjected to radio frequency and microwave energies. For nondiapausing larvae, the treatment temperatures (44–52°C) and exposure times (0.1–120 min) used were based on the thermal-death-time (TDT) curve for fifth-instar codling moth (Ikediala et al. 2000) and navel orangeworm (Wang et al. 2002b). At each temperature, 4–9 exposure times were selected to provide a wide range of mortality levels, including 100%. Nondiapausing control larvae were placed in the unheated block chamber for 120 min, equivalent to the longest treatment exposure. Because fewer diapausing larvae were available for treatment, they were treated at just four different temperature-time combinations: 46°C for 10 and 17 min, 48°C for 7 min, and 50°C for 2 min. Diapausing control larvae were placed in the unheated block chamber for 50 min. For each temperature and exposure, including controls, 200 larvae were treated at a time and all treatments were repeated three times for a total of 600 larvae.

At the end of each exposure, the power to the heating block was turned off and the insects were quickly (within 10 s) transferred to a plastic container at room temperature. Cardboard strips were provided for resting sites. Because we anticipated that commercial treatments would include rapid post-treatment cooling of product to minimize the effect on product quality, the treated larvae were immediately moved to cold storage at 4°C and stored at this temperature for 1 d. After the cold storage, the larvae were held at 23°C, 60% RH and a 14:10 (L:D) h photo-period for 1 d to minimize the effect of cold stupor before examination. Insects were considered dead if the body was dark or dried out. Moribund and surviving larvae were observed for an additional 5 d.

Table 1. Coefficients of determination (r^2) from kinetic order (n) models for thermal mortality of fifth-instar Indianmeal moth at five temperatures

Temperature	N_t*	n = 0	n = 0.5	n = 1	n = 1.5	n = 2
44	4,200	0.979	0.963	0.628	0.358	0.330
46	5,400	0.866	0.962	0.695	0.383	0.349
48	2,400	0.905	0.990	0.815	0.574	0.541
50	2,400	0.911	0.958	0.924	0.688	0.630
52	2,400	0.975	0.938	0.679	0.476	0.453

* N_t = Total number of test insects per temperature; 200 insects for each of 3 replications and 4–9 exposures.

Treatment Model and Statistical Analysis. Survival was calculated as the proportion of surviving larvae relative to total treated larvae for each treatment. Mean values and standard deviations were obtained from three replications for each temperature-time combination. In developing the models, a value of 0.1 was used for the number of surviving insects where actual survival was 0%.

We used both an empirical model (Alderton and Snell 1970) and a fundamental kinetic model to describe the response of nondiapausing larvae to heat. The equation for the empirical model is as follows:

$$\left(\log \frac{N_0}{N}\right)^a = kt + c \qquad [1]$$

where N_0 and N are the initial and surviving numbers of insects, t is the exposure time (min) at a fixed temperature, k is the thermal death rate constant (1/min), a and c are constants. A more detailed explanation of the development of the empirical model is found in Wang et al. (2002b). The model was used to estimate the LT_{95} and LT_{99} for each treatment temperature.

The kinetic model is similar to that previously used for codling moth (Wang et al. 2002a) and navel orangeworm (Wang et al. 2002b), and is based on the following equation:

$$\frac{d(N/N_0)}{dt} = -k(N/N_0)^n \qquad [2]$$

where n is the kinetic order of the reaction. The integration form of equation 2 can be obtained for different reaction orders as follows:

$$\begin{aligned} \ln(N/N_0) &= -kt + c \quad (n = 1) \\ (N/N_0)^{1-n} &= -kt + c \quad (n = 1) \end{aligned} \qquad [3]$$

For each temperature, survival (N/N_0) was regressed against exposure time (t) according to equation 3 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 nondiapausing larvae was developed by plotting for each temperature the minimum exposure time required to achieve 100% mortality of test larvae on a semilog scale (Ikediala et al. 2000, Wang et al. 2002b). The z value (the temperature difference by 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):

$$E_a = \frac{2.303RT_{\min}T_{\max}}{z} \qquad [4]$$

where R is the universal gas constant (8.314 J/mol K), $T_{\min}$ and T_{max} are the minimum and maximum temperatures (K) of a test range, respectively. Activation energy for thermal death of test larvae was also 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:

$$k = k_{ref}e^{\frac{-Ea}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)} \qquad [5]$$

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

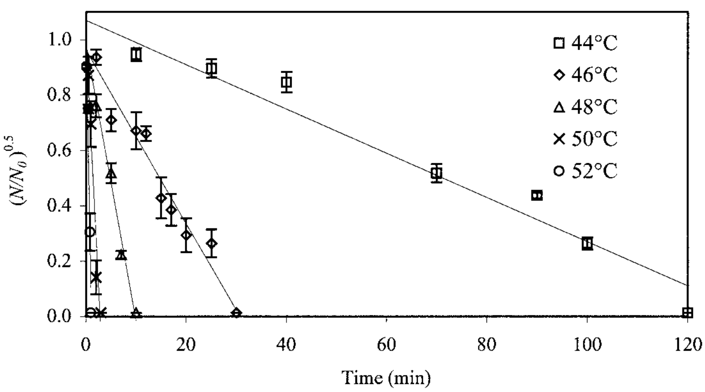

Fig. 1. Thermal mortality curves of fifth-instar Indianmeal moth at different temperatures. N_0 = number of treated insects (600), N = number of surviving insects.

Table 2. Empirical and kinetic model parameters (±SE) for thermal response of fifth-instar Indianmeal moth at five temperatures

Temperature (°C)	N_t*	Empirical model $\left(\log \frac{N_0}{N}\right)^a = kt + c$				0.5th order kinetic model $(N/N_0)^{0.5} = -kt + c$		
		a	k	c	r^2	k	c	r^2
44	4,200	0.397	0.0114 ± 0.0014	0.0498 ± 0.164	0.92	0.0078 ± 0.0006	1.069 ± 0.045	0.96
46	5,400	0.397	0.0445 ± 0.0050	0.1798 ± 0.148	0.91	0.0313 ± 0.0022	0.963 ± 0.036	0.96
48	2,400	0.397	0.1567 ± 0.0157	0.0816 ± 0.124	0.97	0.0999 ± 0.0059	0.983 ± 0.035	0.99
50	2,400	0.397	0.5537 ± 0.0304	0.0786 ± 0.073	0.99	0.3595 ± 0.0434	1.011 ± 0.073	0.96
52	2,400	0.397	1.4323 ± 0.2615	0.0456 ± 0.226	0.91	0.9406 ± 0.1397	1.046 ± 0.861	0.94

* N_t = Total number of test insects per temperature; 200 insects for each of 3 replications and 4–9 exposures.

Treatment mortality for nondiapausing and diapausing larvae was compared for each temperature-time combination using the SAS *t*-test (TTEST) procedure (SAS Institute 1989). We used an arcsine transformation to normalize the data before analysis.

Results and Discussion

Thermal Death of Nondiapausing Fifth-Instar Larvae. Shipping temperatures varied from 7.5 to 30°C over the 18 h of transit time, and were not considered to have a negative effect on test insects. Mortality of untreated nondiapausing larvae was low (1.5 ± 0.35%), suggesting that shipping and handling had little effect on test insects. As such, mortality data for treated larvae were not corrected for control mortality.

Coefficients of determination (r^2) derived for each kinetic order model and treatment temperature are given in Table 1. As we found for codling moth (Wang et al. 2002a) and navel orangeworm (Wang et al. 2002b), the 0.5th order reaction (Fig. 1) produced the most consistently high r^2 values (≥0.938) for all temperatures. Consequently, we chose this model for further calculations.

Model parameters for the empirical model and the 0.fifth order kinetic model for each treatment temperature are given in Table 2. Estimates of LT_{95} and LT_{99} for both models and the observed minimum exposure resulting in 100% mortality are compared in Table 3. For all treatment temperatures, estimates of LT_{95} and LT_{99} from the two models were similar, judging by overlapping 95% CIs. However, estimates from the kinetic model were consistently higher than those from the empirical model, indicating that the kinetic model would provide more conservative treatment protocols.

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. As shown above, the fundamental kinetic method provides precision similar to that of the modified logarithmic model, is considerably easier to calculate, and results in more conservative treatment times. Similar results were found for codling moth (Wang et al. 2002a) and navel orangeworm (Wang et al. 2002b). Consequently, the remainder of the discussion will be based on estimates derived from the kinetic model.

For treatment temperatures of 44 and 46°C, exposures of 106 and 24 min, respectively, were required to produce 95% mortality (LT_{95}). Such lengthy exposures would not be appropriate for commercial applications, which must use continuous material flow to handle large amounts of product. Treatment temperatures of 50 and 52°C would be more practical, as these produced 95% mortality after exposures of just two and 1 min, respectively. Such temperatures can be obtained easily in walnuts with radio frequency energy (Wang et al. 2001, 2002c).

Nondiapausing Indianmeal moth larvae were less heat tolerant than either codling moth or navel orangeworm. Codling moth larvae required exposure times twice those for Indianmeal moth for 100% mortality at comparable temperatures (Wang et al. 2002a). Exposure times needed for 100% mortality of navel orangeworm were five times longer than for Indianmeal moth (Wang et al. 2002b). Consequently, any treatment designed to disinfest product of navel or-

Table 3. Comparison of lethal times (min) calculated by empirical and kinetic models and observed exposure to achieve 100% mortality for fifth-instar *P. interpunctella* at five temperatures

Temperature (°C)	N_t*	Observed minimum for 100% mortality of 600 insects	Empirical model				0.5th order kinetic model			
			LT_{95}	95% CI	LT_{99}	95% CI	LT_{95}	95% CI	LT_{99}	95% CI
44	4,200	120	93.0	77.5–108.5	111.1	92.0–130.2	105.9	93.7–118.1	121.4	106.8–136.0
46	5,400	30	20.9	18.0–23.8	25.5	21.9–29.1	23.6	21.5–25.7	27.5	24.9–30.1
48	2,400	10	7.0	5.8–8.2	8.3	6.8–9.8	7.6	6.7–8.4	8.8	7.8–9.8
50	2,400	3	1.9	1.7–2.1	2.2	1.9–2.5	2.2	1.6–2.7	2.5	1.9–3.1
52	2,400	1	0.7	0.5–1.0	0.9	0.6–1.2	0.9	0.7–1.3	1.0	0.7–1.3

* N_t = Total number of test insects per temperature; 200 insects for each of 3 replications and 4–9 exposures.

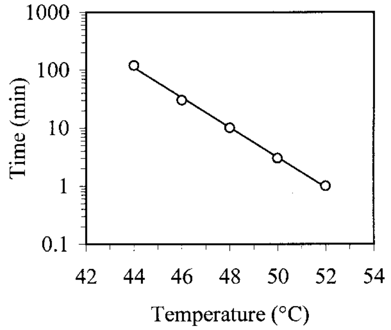

Fig. 2. TDT curve for fifth-instar Indianmeal moth (N = 600) at a heating rate of 18°C/min. Line represents linear regression equation $\log t = 13.39–0.26\,T$ ($r^2 = 0.99$) where t = time and T = temperature.

angeworm should also be effective against nondiapausing Indianmeal moth.

Thermal Death Time Curve and Activation Energy. A thermal death time (TDT) curve for fifth-instar Indianmeal moth is given in Fig. 2. The curve defines minimum time-temperature combinations to achieve 100% mortality in a sample of 600 insects. The curve is described by the linear regression equation $\log t = 13.39 - 0.26\,T$ ($r^2 = 0.99$) where t = time and T = temperature. The z value derived from the TDT curve is 3.9°C, and yields an activation energy for thermal death of fifth-instar Indianmeal moth of 506.3 kJ/mol. An alternative method to calculate activation energy from the slope of an Arrhenius plot (Fig. 3) for rate constant, k, resulted in an estimation of 513.7 kJ/mol.

The activation energy is useful in determining the sensitivity of insects to changes in temperature. The activation energy for thermal death of fifth-instar Indianmeal moth calculated from the TDT curve was lower than that found for navel orangeworm (519 kJ/mol) by Wang et al. (2002b) but slightly higher than that for codling moth (499 kJ/mol) calculated by Ikediala et al. (2000). This value was within the range of the activation energies calculated for eggs and larvae of tephritid fruit flies (400–957 kJ/mol) (Jang 1986).

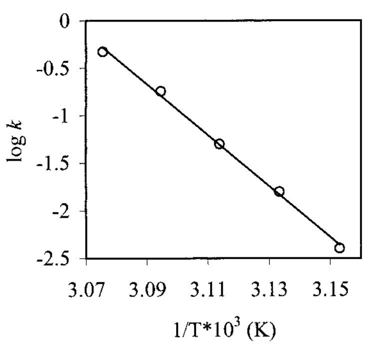

Fig. 3. Arrhenius plot for temperature effects on thermal death rates of fifth-instar Indianmeal moth. Line represents linear regression equation $\log k = 82.22–26.83*1000/T$ (r^2 = 0.99) where t = time and T = temperature.

Table 4. Comparative heat tolerance of non-diapausing and diapausing fifth-instar Indianmeal moth larvae (N = 600)

Temp (°C)	Exposure (min)	% Mortality ± SE	
		Nondiapausing larvae	Diapausing larvae
25	50	1.0 ± 0.58 NS	0.7 ± 0.33 NS
46	10	54.9 ± 9.10**	9.2 ± 3.06**
	17	85.1 ± 4.62 NS	79.8 ± 4.64 NS
48	7	95.0 ± 0.58 NS	91.4 ± 7.62 NS
50	2	98.0 ± 1.32*	83.3 ± 6.41*

t test preformed on arcsine transformed data, *, $P \leq 0.05$, **, $P \leq 0.01$; NS, not significant (SAS Institute 1989).

Heat Tolerance of Diapausing Larvae. Control mortality for both diapausing and nondiapausing fifth-instar Indianmeal moth larvae was very low, and differences between the two groups were insignificant (Table 4). At all time-temperature treatment combinations, mortality for diapausing larvae was lower than that for nondiapausing larvae. However, significant differences were detected at only two treatments: 46°C for 10 min ($t = -4.93$; df = 4; $P = 0.0079$) and 50°C for 2 min ($t = -2.80$; df = 4; $P = 0.049$). Differences between diapausing and nondiapausing larvae appeared to decrease as treatment severity increased. At the target temperatures suggested for radio frequency treatments (50–55°C), there may not be a noticeable difference between diapausing and nondiapausing larvae. Moreover, diapausing Indianmeal moth larvae were less heat tolerant than fifth-instar navel orangeworm (Wang et al. 2002b), and any treatment designed to control navel orangeworm should also be effective against both diapausing and nondiapausing Indianmeal moth.

Acknowledgments

This research was supported by grants from USDA-NRI (99-35316–8099) and USDA IFAFS (00-52103–9656) programs. We thank Karen Valero (USDA-ARS, Parlier, CA) for her technical assistance, and Frank Arthur (USDA-ARS, Manhattan, KS) and James Hansen (USDA-ARS, Wapato, WA) for reviewing the manuscript.

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Received for publication 14 August 2002; accepted 6 November 2002.