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RESEARCH

Thermal Death Kinetics of Fifth-Instar *Corcyas cephalonica* (Lepidoptera: Galleriidae)Liling Yang,^{1,2} Li Zhongxin,^{1,3} Wenqiang Ma,¹ Shengkun Yan,¹ and Kuanbo Cui¹¹Agricultural Mechanization Institute, Xinjiang Academy of Agricultural Sciences, No. 291 North Nanchang Rd., Urumqi, Xinjiang, China²College of Engineering, China Agricultural University, No. 17 East Tsinghua Rd., Beijing, China³Corresponding author, e-mail: xjxn669@163.com

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ABSTRACT. The infestation of rice moth, *Corcyas cephalonica* (Lepidoptera: Galleriidae), causes severe losses in postharvest walnuts. Heat has been studied as a phytosanitary treatment to replace chemical fumigation for controlling this pest. Information on kinetics for thermal mortality of *C. cephalonica* is needed for developing effective postharvest phytosanitary thermal treatments of walnuts. Thermal death kinetics of fifth-instar *C. cephalonica* were investigated at temperatures between 44°C and 50°C at a heating rate of 5°C min⁻¹ using a heating block system. The results showed that thermal-death curves for *C. cephalonica* larvae followed a 0 order of kinetic reaction. The time to reach 100% mortality decreased with increasing temperature from 150 min at 44°C to 2.5 min at 50°C. The activation energy for controlling *C. cephalonica* was 466–592 kJ/mol, and the z value obtained from the thermal death time curve was 3.3°C. This kinetic model prediction could be useful in designing the thermal treatment protocol for controlling *C. cephalonica* in walnuts.

Key Words: thermal death time, kinetics, heating block, heating rate, rice moth

Walnut (*Juglans regia* L.) is one of important nuts with high economic values to the local agriculture. The world production of in-shell walnuts was around 3.282 Mt in 2012, and China is the leading world producer with about 50% of the total world production (Food and Agriculture Organization Statistics Database [FAOSTAT] 2012). During harvesting, processing, and storage, however, the infestation of the rice moth, *Corcyas cephalonica* (Lepidoptera: Galleriidae), is the largest problem in Chinese walnuts, causing product quality losses through feeding damage and by contaminating the product with webbing (Wang et al. 2007a,b). Currently, many processors are using methyl bromide fumigations to disinfest walnuts of *C. cephalonica* (Aegerter and Folwell 2001). According to the Montreal Protocol (United States Environmental Protection Agency [USEPA] 2001), the global use of methyl bromide will be phased out by 2015 in developing countries, such as China, due to its listing as an ozone depleter (USEPA 2001). Therefore, there is an urgent need to develop an alternative nonchemical phytosanitary method to completely control the *C. cephalonica* in walnuts.

Heating with radio frequency (RF) energy has been proposed as a potential alternative to chemical fumigation for postharvest control of insects in agricultural products (Tang et al. 2000). A laboratory scale RF system was used to evaluate the potential of RF heat treatments to control insect pests in California walnuts (Wang et al. 2001, 2002c). After that, a 25 kW industrial-scale RF system was successfully used to control fifth-instar navel orangeworms, *Amelois transitella*, in in-shell walnuts with a throughput of 1,560 kg/h without adverse effects on product quality after treatments and 2-yr storage (Wang et al. 2007a,b). Thus, the RF heating holds potential to be a practical alternative to chemical fumigation for disinfesting Chinese walnuts.

Developing a successful thermal treatment protocol using RF energy relies on a thorough knowledge of the thermal death kinetics of the targeted insect in walnuts. Experimental methods for characterizing the temperature–time effect on insect mortality include directly exposing insects in a water bath, 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, Hansen and Sharp 2000, Waddell et al. 2000). To provide more accurate and uniform temperature control for experiments, a heating block system (HBS) has been developed to study the thermal death kinetics of several important insect pests (Wang et al.

2002a,b; Johnson et al. 2003, 2004; Gazit et al. 2004; Hallman et al. 2005). The HBS results have been confirmed with RF heat treatments for walnuts infested with fifth-instar codling moth, *Cydia pomonella* (Wang et al. 2001a) and *A. transitella* (Wang et al. 2002c, Mitcham et al. 2004), and hot water treatments for cherries infested with third-instar codling moth (Feng et al. 2004; Hansen et al. 2004). The reliable and repeatable results obtained from the HBS demonstrate that the latest instars were the most heat-tolerant life stage of the targeted insects. Yokoyama et al. (1991) also observed that the fifth instar was the most heat-resistant life stage at 49–51°C using water heating. Thus, we selected fifth-instar *C. cephalonica* for this initial study.

Thermal death kinetic models derived from thermal mortality data may provide useful information on predicting the efficacy of different treatment conditions tested and guiding new treatment protocol developments. Although only 0th order kinetic model is reported for adult rice weevil, *Sitophilus oryzae* (Yan et al. 2014), the 0.5th order kinetic model has been mostly applied to thermal response of many insects, such as *Cy. pomonella* (Wang et al. 2002a), Indianmeal moth, *Plodia interpunctella* (Johnson et al. 2003), *A. transitella* (Wang et al. 2002b), Mediterranean fruit fly, *Ceratitis capitata* (Gazit et al. 2004, Armstrong et al. 2009), red flour beetle, *Tribolium castaneum* (Johnson et al. 2004), and Mexican fruit fly, *Anastrepha ludens* (Hallman et al. 2005). To develop effective heat treatments on disinfesting walnuts, it is essential to determine the thermal death kinetic models for the *C. cephalonica* based on the HBS data.

The objectives of this research were to 1) determine the thermal mortality of fifth-instar *C. cephalonica* at four selected temperatures as a function of holding time using the HBS, 2) develop the thermal death kinetic model of the *C. cephalonica* larvae, 3) predict the holding time needed to achieve the required mortality at given populations, and 4) compare the activation energy for controlling insects in this study with those for other pests in the literature.

Materials and Methods

Heating Block System (Fig. 1). An HBS consisting of two aluminum blocks (254 by 254 by 18 mm³) built by Washington State University was rented from Northwest A&F University and used to heat the fifth-instar *C. cephalonica* (Ikediala et al. 2000, Wang et al. 2002b).

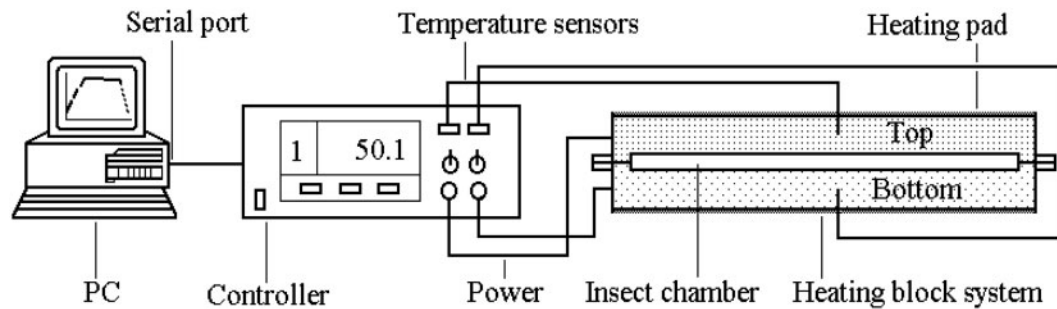


Fig. 1. HBS for insect mortality studies (Yin et al. 2006).

Table 1. Heating temperatures and exposure times used in thermal death kinetic tests

Temperature (°C)	Holding time (min)				
44	30	60	90	120	150
46	10	20	30	40	50
48	3	6	9	12	15
50	0.5	1	1.5	2	2.5

When placed together, the blocks formed a 3-mm thick close chamber where the insects were placed. Four electric heating pads (250 W each) were attached to the back of each block to provide total thermal energy of $15,500 \text{ Wm}^{-2}$. Calibrated type T thermocouples inserted through holes near the center of each block monitored temperatures. Heating rate, set-point temperature, and exposure time were controlled by a customized Visual Basic program and PID controllers (I32, Omega Engineering Inc., Stamford, CT) via a solid state relay. The high thermal capacitance of the blocks provided smooth temperature profiles over the heating and holding periods with temperature deviations from the set point ($\leq 60^\circ\text{C}$) of $<0.3^\circ\text{C}$ (Wang et al. 2002b). A detailed description of the HBS could be found in Ikediala et al. (2000) and Wang et al. (2002b).

Test Insect. Fifth-instar *C. cephalonica* were reared at Institute of Plant Protection, Xinjiang Academy of Agricultural Sciences, Urumqi, Xinjiang, China, on a wheat bran and corn flour diet at 27°C , 60% relative humidity (RH), and a photoperiod of 14:10 (L:D) h. Actively moving larvae were chosen for each test.

Treatment Procedures. Based on the thermal death time (TDT) curves for *P. interpunctella* (Johnson et al. 2003), *T. castaneum* (Johnson et al. 2004), and *S. oryzae* (Yan et al. 2014), five exposure times (0.5–150 min) at 44, 46, 48, and 50°C (Table 1) were selected to provide a wide range of mortality levels up to 100% for *C. cephalonica* larvae. A $5^\circ\text{C}/\text{min}$ was used to simulate the fast heating rate for microwave or RF heating in walnuts (Wang et al. 2007a,b). The HBS was preheated to room temperature (25°C) before test. Fifty actively moving larvae were randomly selected and placed in the insect chamber of the HBS for each temperature–time combination test. Immediately upon completion of the holding time, the top block was opened and held at an elevated angle to allow larvae to roll into plastic containers. Any larvae that did not roll off were immediately removed by delicate brushing. Each temperature/time combination was replicated three times. Control insects were placed on the heating block at 25°C for 150 min. After each test, the HBS was cooled down to 25°C using ice packs and ready for next experiment.

Mortality was determined with 5-day observations after treatment because death was not always immediately obvious. If the larva had turned dark gray, had not initiated pupariation, or would not respond to gentle prodding, it was considered dead. Each test was repeated three times. Mean values and standard deviations were calculated from three replicates for each treatment.

Thermal Death Kinetics Modeling. Mean survival ratios as a function of exposure times at each of the four treatment temperatures were used to develop a thermal death kinetic model based on the basic kinetic equation used for the other stored insects (Johnson et al. 2003, 2004):

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

where N_0 and N are the initial and surviving numbers of larvae, t is exposure time (min), k is the thermal death rate constant (min^{-1}), and n is the kinetic order of the reaction. Where survival was 0%, a value of 0.16% was used for N/N_0 to avoid zero in the numerator. The integrated form of equation (1) can be obtained for different reaction orders as follows:

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

where k and c are the slope and intercept of the regression curve with vertical axis $(N/N_0)^{1-n}$, when plotting against time t .

For each temperature, survival (N/N_0) was regressed against exposure time (t) using the 0-, 0.5th-, 1st-, 1.5th-, and 2nd-reaction order according to equation (2). The reaction order with the largest mean coefficient of determination (R^2) across all four temperatures should be selected as the most suitable for further calculations. Upon selection of the reaction order, values of k and c were derived by regression. After the reaction order was determined, and the values of k and c were obtained, and the model was used to estimate the time needed to kill 95, 99, 99.33, and 99.99% of the test insects (LT_{95} , LT_{99} , $LT_{99.33}$, and $LT_{99.99}$, respectively). After that, the TDT curve for fifth-instar *C. cephalonica* was developed by plotting the minimum exposure time required at each temperature to achieve 100% kill of test larvae on a semilog scale. This type of plot was used for thermal kinetics of *Cy. pomonella* by Wang et al. (2002a). The z value (the temperature difference by which the mortality rate is altered by a factor of 10) was obtained from the TDT curve as described in details elsewhere (Tang et al. 2000).

As the thermal death activation energy (E_a in Joules per mole) of an organism is a determination of its sensitivity to changes in temperature, higher activation energy denotes higher sensitivity. E_a of fifth-instar *C. cephalonica* was determined comparing two independent methods. In one method, E_a was calculated by the following equation from the TDT curve (Tang et al. 2000):

$$E_a = \frac{2.303RT_{\min} T_{\max}}{z} \quad (3)$$

where R is the universal gas constant (8.314 J/mol K), T_{\min} and T_{\max} are the minimum and maximum absolute temperatures ($^\circ\text{K}$), respectively, of a test range, and z is the negative inverse of the slope of the TDT curve ($^\circ\text{C}$).

Table 2. Coefficients of determination (R^2) from kinetic order (n) models for thermal mortality of fifth-instar *C. cephalonica* at four temperatures

Temperatures (°C)	R^2 for different order n				
	$n = 0$	$n = 0.5$	$n = 1$	$n = 1.5$	$n = 2$
44	0.981	0.930	0.904	0.851	0.794
46	0.925	0.961	0.990	0.969	0.914
48	0.989	0.926	0.895	0.834	0.775
50	0.948	0.967	0.933	0.889	0.828
Mean	0.961	0.946	0.931	0.886	0.828

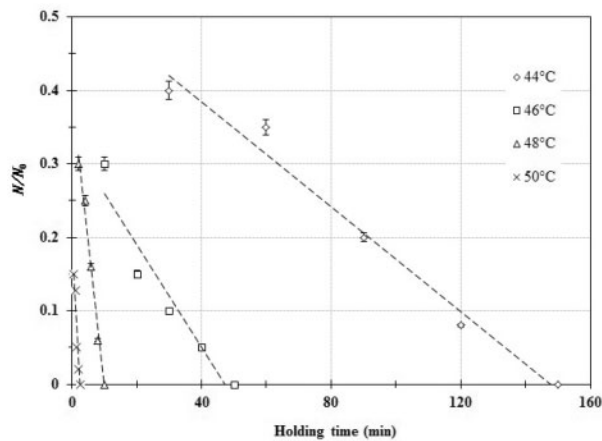


Fig. 2. Thermal mortality curves of fifth-instar *C. cephalonica* at different temperatures and exposures.

Table 3. Thermal death constants of 0th-order reaction model for fifth-instar *C. cephalonica* at four temperatures

Temperature (°C)	$(N/N_0)^{1-0} = -kt + c$	
	k	c
44	0.0030 ± 0.0004	0.4892 ± 0.0132
46	0.0102 ± 0.0017	0.4021 ± 0.0168
48	0.0344 ± 0.0033	0.3688 ± 0.0066
50	0.0764 ± 0.0120	0.1882 ± 0.0060

Table 4. Comparison of LTs (min) obtained by experiments and 0th-order kinetic models for fifth-instar *C. cephalonica* at four temperatures

Temperature (°C)	N_0	Minimum time (min) for 100% mortality of 150 insects	Predicted treatment time (min)			
			LT ₉₅	LT ₉₉	LT _{99.33}	LT _{99.99}
44	150	150	146.4	159.7	160.8	163.1
46	150	50	34.5	38.4	38.8	39.4
48	150	10	9.3	10.4	10.5	10.7
50	150	2.5	1.8	2.3	2.4	2.5

The second method for calculating E_a was through the slope of an Arrhenius plot of $\log k$ versus the reciprocal of the absolute temperature ($1/T$) as follows:

$$\text{Log } k = \text{Log } k_0 - \frac{E_a}{2.303RT} \quad (4)$$

where k_0 is the reference thermal death rate constant (min^{-1}).

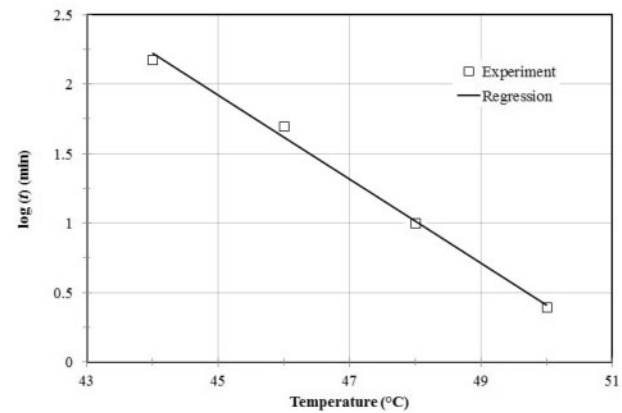


Fig. 3. Thermal mortality curve for fifth-instar *C. cephalonica* at a heating rate of 5°C/min. The fitted equation was $\log t = 15.497 - 0.302 T$ with the coefficient of determination $R^2 = 0.995$.

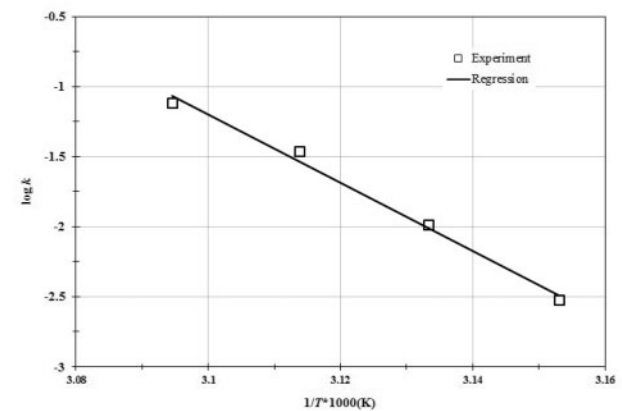


Fig. 4. Arrhenius plot for temperature effects on thermal death rates of fifth-instar *C. cephalonica*. The fitted equation was $\log k = 74.228 - 24.331 \times 1/T \times 1,000$ with the coefficient of determination $R^2 = 0.992$.

Results and Discussion

Thermal Death Kinetics of Fifth-Instar *C. cephalonica*. The average survival rate for unheated controls at the four temperatures was $95.7 \pm 0.3\%$, showing that effects of handling on mortality were negligible. Consequently, data for the heated larvae did not need to be adjusted to compensate for nontreatment mortality. Table 2 lists coefficients of determination (R^2) for different reaction orders at four treatment temperatures to control the fifth-instar *C. cephalonica*. Although the 0.5th and the first-order models yielded the highest R^2 for some individual temperatures, the 0th order had the largest average coefficient of determination over the four temperatures (Table 2) and thus was selected for further model development. This reaction order results were different from 0.5th-order applicable for *An. ludens* (Hallman et al. 2005), *T. castaneum* (Johnson et al. 2004), and *Cy. pomonella* (Wang et al. 2004) but in good agreement with the 0th-order model for adult *S. oryzae* (Yan et al. 2014).

The thermal mortality curves of fifth-instar *C. cephalonica* with the best-fit 0th-order model are shown in Fig. 2. The slopes of the thermal mortality curves increased sharply when the treatment temperature increased from 44 to 50°C. The minimum holding time for 100% mortality of 150 insects at 44, 46, 48, and 50°C was about 150, 50, 10, and 2.5 min, respectively. These lethal times (LTs) are slightly smaller than those for adult *S. oryzae* at 46–50°C (Yan et al. 2014), indicating that a

Table 5. Comparisons of activation energies for thermal kill of insects and microorganisms with that for food quality changes due to heat treatments

Insects	Temperature range (°C)	Activation energy (kJ mol ⁻¹)	Source
Caribbean fruit fly (eggs)	43–50	445	Moss and Chan (1993)
Codling moth (fifth instar)	46–52	472–473	Wang et al. (2002a)
Indianmeal moth (fifth instar)	44–52	514	Johnson et al. (2003)
Mediterranean fruit fly (larvae)	45–48	656	Jang (1986)
	46–52	552	Gazit et al. (2004)
	44–50	648–666	Armstrong et al. (2009)
Melon fly (larvae)	45–48	650	Jang (1986)
	44–50	505–507	Armstrong et al. (2009)
Navel orangeworm (larvae)	46–54	510–520	Wang et al. (2002b)
Oriental fruit fly (larvae)	43–48	209–401	Jang (1986)
	44–50	641–675	Armstrong et al. (2009)
Queensland fruit fly (eggs)	42–48	538	Waddell et al. (2000)
Red flour beetle (third instar)	48–52	814	Johnson et al. (2004)
Rice weevil (adult)	44–50	505–523	Yan et al. (2014)
Quality (texture—softening or firmness, color, etc.)	50–70	42–126	Lund (1977) and Rao and Lund (1986)
Microorganisms (spores)	100–130	222–502	Lund (1977)
Rice moth (fifth instar)	44–50	592	This study from TDT curve
		466	This study from $k-T$ curve

heat phytosanitary treatment efficacious for *S. oryzae* would control *C. cephalonica*. Similarly, the thermal death rate constant k increased with temperature, resulting in that higher temperatures require shorter exposures to achieve the same insect mortality (Table 3). But the constant c was not close to the ideal value of 1 for all four temperatures, suggesting that the mortality due to handling at the room temperature and during the temperature increase phase was not negligible and would little influence the prediction precision of the obtained kinetic model under high temperatures, such as 48 or 50°C.

Table 4 lists the minimum time for 100% mortality of 150 insects and predicted LT to obtain 95, 99, 99.33, and 99.99% mortality. The predicted LTs increased with increasing insect mortality levels. The observed minimum time for 100% mortality was close to the estimated LT_{99.33} at 48 and 50°C but with over estimations at 44°C and under estimations at 46°C. For high-temperature short time treatments, such as RF heating, the established kinetic model could be reliable for mortality predictions of fifth-instar *C. cephalonica* in walnuts.

Figure 3 plots the minimum times to achieve 100% mortality of 150 fifth instars on a semilog scale versus temperature. The z value derived from the negative inverse of the slope of the TDT curve was 3.3°C. This means that every 3.3°C increase in temperature leads to a 10-fold decrease in time required to reach the same level of mortality. This z value was similar to that (3.3°C) for *Ce. capitata* (Armstrong et al. 2009), smaller than that (3.9–4.4°C) for *Cy. pomonella*, *P. interpunctella*, *S. oryzae*, and *A. transitella* (Wang et al. 2002a,b; Johnson et al. 2003; Yan et al. 2014) but slightly larger than that (2.4°C) for *T. castaneum* (Johnson et al. 2004).

Activation Energy. The thermal death activation energy value (E_a) was 592.0 kJ/mol from equation (3), which was larger than that (465.9 kJ/mol) from equation (4) based on the slope of the Arrhenius plot (Fig. 4). This difference was probably caused by the estimated kinetic constant from narrow mortality data (about 30–40%) in the tests.

Table 5 summarizes comparisons of activation energies for thermal kill of insects and microorganisms with that for food quality changes due to heat treatments. Insects with high activation energy levels are more sensitive to changes in temperature. Activation energies were different from the selected insects or heating methods and ranged from 209 to 814 kJ mol⁻¹ for insects. The E_a values for *C. cephalonica* were similar to those for *S. oryzae* (Yan et al. 2014), fifth-instar *Cy. pomonella* (Wang et al. 2002a), fifth-instar *P. interpunctella* (Johnson et al. 2003), fifth-instar *A. transitella* (Wang et al. 2002b), third-instar *Ce. capitata* (Gazit et al. 2004), and Queensland fruit fly, *Bactrocera tryoni* eggs (Waddell et al. 2000), larger than those Caribbean fruit fly, *Anastrepha suspensa* eggs (Moss and Chan 1993), but smaller than those for *T. castaneum* (Johnson et al. 2004) and *Ce. capitata* in Hawaii

(Armstrong et al. 2009). In general, insects are more susceptible to increased temperatures than commodities because the activation energy for thermal kill of insects is slightly greater than that for product quality changes such as texture softening or thermal inactivation of pathogenic microbial spores (Table 5). This provides an opportunity for the possible development of relatively high-temperature/short-time thermal treatment processes that may kill insects while having minimal impact on product quality (Tang et al. 2000).

A validated HBS was used to study the thermal mortality of *C. cephalonica* larvae. The results showed that the 0th-order reaction model could accurately describe the response of *C. cephalonica* larvae to high temperatures. The kinetic parameters, including activation energy, derived from this model were useful in developing effective thermal treatment protocols and comparing relative thermotolerance with other species. The thermal death kinetic models not only allowed prediction of insect mortality with treatment times when subjected to steady-state isothermal heating but could also be incorporated in other models to predict insect thermal mortality under transit and nonisothermal conditions. This thermal-death kinetic information might be used to guide the development of thermal methods for postharvest control of *C. cephalonica* larvae in other agricultural products.

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