Thermal Death Kinetics of Fifth-Instar *Corcyras cephalonica* (Lepidoptera: Galleriidae)

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**ABSTRACT.** The infestation of rice moth, *Corcyras 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, *Corcyras 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 fumigation to completely control the *C. cephalonica* in walnuts. The infestation of rice moth, *Corcyras 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.

**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).
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 W m\(^{-2}\). 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., Stanford, 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 (<0.3°C) of <0.3°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°C/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.

### 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  |

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):

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

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:

\[
\ln \left(\frac{N}{N_0}\right) = -kt + c \quad (n = 1) \tag{2}
\]

\[
\left(\frac{N}{N_0}\right)^{-n} = -kt + c \quad (n \neq 1)
\]

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 (LT95, LT99, LT99.33, and LT99.99, 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 *Cp. 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):

\[
E_a = \frac{2.303RT_{\text{min}}T_{\text{max}}}{z} \tag{3}
\]

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

$$\log k = \log k_0 - \frac{E_a}{2.303RT}$$

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

### 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 ± 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

![Fig. 2. Thermal mortality curves of fifth-instar C. cephalonica at different temperatures and exposures.](image)

| Temperature (°C) | (N/N₀)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 |

![Fig. 3. Arrhenius plot for temperature effects on thermal death rates of fifth-instar C. cephalonica. The fitted equation was log $k = 74.228 - 24.331 \times 1/T*1,000$ with the coefficient of determination $R^2 = 0.992$.](image)

![Fig. 4. Thermal mortality curve for fifth-instar C. cephalonica at a heating rate of 5°C/min. The fitted equation was log $t = 15.497 - 0.302T$ with the coefficient of determination $R^2 = 0.995$.](image)

**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) | $n = 0$ | $n = 0.5$ | $n = 1$ | $n = 1.5$ | $n = 2$ |
|-------------------|--------|----------|--------|----------|--------|
| 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  |

**Table 3. Thermal death constants of 0th-order reaction model 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         |

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

**Results and Discussion**
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 thermal death kinetic models not only allowed prediction of insect mortality with treatment times when subjected to steady-state temperatures. The kinetic parameters, including activation energy, were 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 nonsothermal 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.

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 *Ea* values for *C. cephalonica* were similar to those for *S. oryzae* (Yan et al. 2014), fifth-instar *C. pomonella* (Wang et al. 2002a), fifth-instar *P. interpunctella* (Johnson et al. 2003), fifth-instar *A. transitella* (Wang et al. 2002b), third-instar *C. 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 *C. 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).

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 nonsothermal 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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