

Thermal Death Kinetics of *Conogethes Punctiferalis* (Lepidoptera: Pyralidae) as Influenced by Heating Rate and Life Stage

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ABSTRACT Thermal death kinetics of *Conogethes punctiferalis* (Guenée) (Lepidoptera: Pyralidae) at different life stages, heating rate, and temperature is essential for developing postharvest treatments to control pests in chestnuts. Using a heating block system (HBS), the most heat-tolerant life stage of *C. punctiferalis* and the effects of heating rate (0.1, 0.5, 1, 5, and 10°C/min) on insect mortality were determined. The thermal death kinetic data of fifth-instar *C. punctiferalis* were obtained at temperatures between 44 and 50°C at a heating rate of 5°C/min. The results showed that the relative heat tolerance of *C. punctiferalis* was found to be fifth instars > pupae > third instars > eggs. To avoid the enhanced thermal tolerance of *C. punctiferalis* at low heating rates (0.1 or 0.5°C/min), a high heating rate of 5°C/min was selected to simulate the fast radio frequency heating in chestnuts and further determine the thermal death kinetic data. Thermal death curves of *C. punctiferalis* followed a 0th-order kinetic reaction model. The minimum exposure time to achieve 100% mortality was 55, 12, 6, and 3 min at 44, 46, 48, and 50°C, respectively. The activation energy for controlling *C. punctiferalis* was 482.15 kJ/mol with the z value of 4.09°C obtained from the thermal death–time curve. The information provided by thermal death kinetics for *C. punctiferalis* is useful in developing effective postharvest thermal treatment protocols for disinfesting chestnuts.

KEY WORDS *Conogethes punctiferalis*, thermal treatment, life stage, heating rate, thermal death kinetics

The *Conogethes punctiferalis* (Guenée) (Lepidoptera: Pyralidae), commonly known as yellow peach moth in China, is a typically polyphagous pest of many orchard crops, plants, and vegetable crops, such as apricot, chestnut, peach, plum, maize, and sunflower (Xiao et al. 2012). The larvae of this species are one of the major pests found in chestnuts. Losses caused by this pest in chestnuts include reduced nutritional value, low percentage germination, and reduced weight (Yuya et al. 2009). Furthermore, the pests also produce silk and frass, which create favorable conditions for mold growth and reduce the market price of chestnuts. At present, *C. punctiferalis* is targeted by quarantine regulations in the USA, Canada, and other countries (Du et al. 2012). Therefore, it is necessary to effectively reduce damage by this pest before chestnuts enter storage or the marketing channel.

Until recently, the most commonly used method for postharvest disinfestation treatment is methyl bromide fumigation because of its rapid action, low cost, and broad spectrum of activity (Yokoyama et al. 1990). With increasing concerns over chemical residues affecting human health, and adverse influences on the environment, many non-chemical alternative methods, such as cold storage, controlled atmosphere, ionizing radiation, low pressure, and thermal treatment, have been proposed to control pests in agricultural products (Fields and White 2002). All of these methods have disadvantages, such as substantial capital investments, lengthy treatment times, or concerns over consumer acceptance. One of the advanced thermal treatment technologies, radio frequency (RF) heating, has been suggested as a promising physical method for disinfestations because of its rapid, volumetric heating, large penetration depth, and lack of chemical residues (Nelson 1996, Piyasena et al. 2003, Jiao et al. 2011, Hou et al. 2014). Industrial-scale RF treatments have been successfully used to control insect pests in walnuts and lentils without negative effects on product quality (Wang et al. 2007a, b; Jiao et al. 2012).

Knowledge of thermal death kinetics for insect pests is essential in developing effective thermal treatments by RF energy. Experimental methods used for studying thermal death kinetics of insects include directly exposing insects in a water bath for predetermined times, heating insects in tubes that, in turn, are submerged in

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a water bath, or heating insects in fruits (Yokoyama et al. 1991, Waddell et al. 2000). To provide repeatable and reliable thermal mortality data of insects, an HBS was developed at Washington State University, Pullman, WA, and used to determine heat tolerance of codling moth, *Cydia pomonella* (L.) (Wang et al. 2002a, 2004), navel orangeworm, *Amyelois transitella* (Walker) (Wang et al. 2002b), Indianmeal moth, *Plodia interpunctella* (Hübner) (Johnson et al. 2003), red flour beetle, *Tribolium castaneum* (Herbst) (Johnson et al. 2004), Mediterranean fruit, *Ceratitis capitata* (Wiedemann) (Armstrong et al. 2009), and rice weevil, *Sitophilus oryzae* (L.) (Yan et al. 2014). Thermal death kinetic data derived with the HBS have been validated by efficacy tests against insect pests in hot water- and RF-treated products (Feng et al. 2004, Wang et al. 2007b). The same HBS could be used in this research to determine the thermal death kinetics of *C. punctiferalis*.

Because the heat sensitivity of different insect developmental stages varies considerably due to various physiological and environmental factors, it is important to determine the most heat-tolerant life stage of target pests for a given product. For example, fifth-instar codling moth was the most heat-tolerant stage (Yokoyama et al. 1991, Wang et al. 2004). Pupae of *S. oryzae* were the most tolerant stage at 25°C and 60% relative humidity (RH) with 65% CO₂ concentrations, while adults were the most susceptible stage (Annis and Morton 1997). The relative heat tolerance of red flour beetle stages was found to be older larvae > pupae and adults > eggs and younger larvae (Johnson et al. 2004). Furthermore, the heating rates may influence the mortality of insects (Evans 1986). Neven (1998) reported that longer exposure times were needed to achieve the same 95% mortality of fifth-instar codling moth at a slower heating rate with the same temperature. The mortality of fifth-instar navel orangeworm at 1°C/min heating rate was significantly lower than that at the heating rate of 10°C/min (Wang et al. 2005, Yin et al. 2006). A similar phenomenon was found for heating rate effects on adult rice weevil (Yan et al. 2014). Therefore, understanding the effect of different life stages and heating rates on the mortality of *C. punctiferalis* is useful in developing effective thermal treatment protocols.

The objectives of this study were 1) to determine the most heat-tolerant life stage of *C. punctiferalis* at three selected temperature–time combinations using the HBS; 2) to explore the effects of heating rates on thermal mortality of the most heat-tolerant life stage (fifth-instar) for *C. punctiferalis*; 3) to determine the thermal mortality of fifth-instar *C. punctiferalis* at four selected temperatures as a function of holding time; 4) to develop the thermal death kinetic model and estimate activation energy of the fifth-instar *C. punctiferalis*; and 5) to propose a practical treatment protocol to control *C. punctiferalis* in chestnuts.

Materials and Methods

Heating Block System. Thermal mortality tests of test insects were conducted using the HBS, which

provided heating rates between 0.1 and 15°C/min. The HBS consisted of top and bottom heating blocks, between which an insect chamber was formed, and a data acquisition/control unit. Temperatures of both heating blocks were measured using type-T thermocouples sensors. Heating rate, set-point temperature, and holding time were controlled by the customized Visual Basic software and PID controllers (i32 temperature & process controller, Omega Engineering Inc., Stamford, CT) via a solid state relay. Detailed information on the HBS can be found elsewhere (Wang et al. 2002a).

Test Insects. Larvae of the *C. punctiferalis* were reared on chestnuts in glass-rearing containers (6 cm in diameter × 9 cm in height) covered by a fine mesh cloth for air exchange, and maintained in a rearing room at 25 ± 1°C, 70–80% RH, and a photoperiod of 16:8 (L:D) h with artificial light. Only actively moving larvae were used in tests. Adults were kept in cages (16 cm in diameter × 32 cm in height) and provided with sponges soaked in a 10% sucrose solution. Eggs were laid on wax paper covered with the mouths of the adult cages. Pupae and eggs were kept at the same environmental conditions. In this study, those life stages, such as eggs, third instars, fifth instars, and pupae, that might be found infesting chestnuts were selected for heat resistance tests.

Treatment Procedures. To determine the most heat-tolerant life stage among eggs, third instar, fifth instar, and pupae of *C. punctiferalis*, three temperature–time combinations at the heating rate of 5°C/min were selected: 46°C for 8 min, 48°C for 4 min, and 50°C for 2 min. Control insects were placed in the unheated block chamber at room temperature (25°C) for 10 min. Including the control, 50 eggs, larvae, or pupae were treated at each temperature–time combination.

When the white eggs of *C. punctiferalis* turned to deep red gradually, usually taking 4–5 d (Honda et al. 1979), the sheets were cut into small pieces. Each piece containing ~50 eggs was then uniformly moistened with tap water and placed on the bottom heating block to ensure a good contact between the wax paper sheets and the block surface before heat treatments. The wax paper was placed with the eggs face down on the block surface. Control eggs were also spread with tap water. After treatment, the eggs were held under rearing conditions for hatching. Egg mortality was calculated as the percentage of unhatched eggs relative to total treated eggs.

To prevent escape of test larvae and allow quick posttreatment removal from the heat block, 50 test larvae were placed in a nylon-mesh bag at the beginning of each treatment. As the insect chamber in the HBS was only 3 mm in height and the heat transfer effect of the bag on the insect mortality was negligible (Yan et al. 2014), the bag was directly put onto the bottom heating block. The top block was then placed on the bottom block and the treatment program began. At the end of each exposure, test insects were transferred to rearing containers. Treated insects were held under rearing conditions until evaluation. Larvae were

evaluated 48 h after treatment and were considered dead if the body was dark or not moving.

When the white pupae turned to deep red, usually taking 3–4 d (Du et al. 2012), 50 pupae were placed directly on the bottom block. At the end of each exposure, test pupae were also transferred to rearing containers. Treated and control pupae were held under rearing conditions until adult emergence, usually taking 6–10 d. All moving adults were considered as survivors.

To determine the effect of heating rate on mortality for the most heat-tolerant life stage of *C. punctiferalis*, heating rates of 0.1, 0.5, 1, 5, and 10°C/min were used. Based on preliminary experiment, a treatment temperature of 46°C with an exposure of 8 min was selected to provide mortality levels of <100%. Heating rates $\leq 1^\circ\text{C}/\text{min}$ simulated hot air and hot water heat treatments and fast heating rates $\geq 5^\circ\text{C}/\text{min}$ simulated rapid heating using RF energy.

The fifth instars, the most heat-tolerant life stage of *C. punctiferalis*, were used in thermal death kinetic tests. Five exposure times (0.5–50 min) at 44, 46, 48, or 50°C were selected to provide a wide range of mortality levels, including 100%. Control insects were placed in the unheated block for 60 min. Approximately 50 test insects were treated at each temperature–time combination, including controls.

Insect Thermal Kinetic Modeling. The mortality change of insect pests is an important index to evaluate the effect of various temperature and time combinations. If N and N_0 are the surviving and initial numbers of pests, the mortality M (%) of pests can be defined as:

$$M = \left(1 - \frac{N}{N_0}\right) \times 100 \quad (1)$$

A fundamental kinetic model was used to describe the response of the most tolerant life stage to heat and to estimate lethal time (LT) at different population levels. The model is similar to that previously used for codling moth (Wang et al. 2002a), navel orangeworm (Wang et al. 2002b), Indianmeal moth (Johnson et al. 2003), and rice weevil (Yan et al. 2014), and is based on the following equation:

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

where t is the exposure time (min) at a given temperature, k is the thermal death rate constant (1/min), and n is the kinetic order of reaction. The integration form of equation 1 can be obtained for different reaction orders as follows:

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

For each temperature, survival ratio (N/N_0) was regressed against exposure time (t) according to equation 3 for the reaction orders of 0, 0.5, 1, 1.5, and 2. 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} , LT_{99} , $LT_{99.33}$, and $LT_{99.99}$ for each treatment temperature.

Activation Energy. A thermal death–time (TDT) curve for the fifth instar was developed by plotting the minimum exposure time required to achieve 100% mortality of test insects at each temperature on a semi-log scale. The z value, 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):

$$E_a = \frac{2.303RT_{\min}T_{\max}}{z} \quad (4)$$

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) as follows (Tang et al. 2000, Wang et al. 2002b):

$$k = k_{ref} e^{-\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{ref}}\right)} \quad (5)$$

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

Statistical Analysis. Each test was repeated three times. Mean values and SDs were calculated from the replicates for all treatments. All statistical analyses were performed at a 5% significance level using the Microsoft Excel variance procedure (Microsoft Office Excel 2007).

Results and Discussion

Heat-Resistant Life Stage. The mortality (mean \pm SD) in unheated controls was 19.15, 4.26, 2.51, and 11.11% for eggs, third instars, fifth instars, and pupae, respectively. The relatively high mortality in the controls for eggs was the result of the difficulty in handling the delicate insects, which was also observed for Mediterranean fruit fly eggs (Gazit et al. 2004) and codling moth eggs (Wang et al. 2004). The control mortality for other life stages was small, and the effect of handling could be negligible. However, differences in control mortality for all four-life stages were significant ($P < 0.05$). All mortality data were corrected for control mortality using the formula proposed by Abbott (1925) for each life stage before further statistical analysis, which are also widely used in literature (Wang et al. 2004, Neven and Rehfield-Ray 2006).

Table 1 shows the mortality of different *C. punctiferalis* life stages after heating at a rate of 5°C/min. Fifth-instar larval mortality after exposure to 46, 48, and

50°C was consistently lower than other stages, while egg mortality was consistently the highest. Generally, the relative heat tolerance of *C. punctiferalis* stages was found to be fifth instar > pupae > third instar > eggs. Similar results were found in confused flour beetle (Oosthuizen 1935), Mediterranean fruit fly (Armstrong et al. 2009), and red flour beetle (Johnson et al. 2004). Although the average mortality for fifth instars was consistently less than that for pupae, the difference was significant only at 46°C + 8 min ($P < 0.05$). As pupae were rarely found in chestnuts, we selected fifth instar for further studies on thermal death kinetics and effects of different heating rates.

Effect of Heating Rate on Thermal Mortality. Figure 1 shows the mortality changes of fifth-instar *C. punctiferalis* at 46°C for 10 min, as influenced by heating rates. The average mortality was similar at heating rates of 1°C/min and above ($P > 0.05$), which was in agreement with the findings in fifth-instar navel orangeworm (Wang et al. 2005) and rice weevil (Yan et al. 2014). However, the mortality at heating rates <1°C/min was significantly reduced from those at higher heating rates ($P < 0.05$), and was in agreement

with observations reported by Yan et al. (2014). The thermal tolerance of *C. punctiferalis* was enhanced at low heating rates for large samples obtained by conventional heating. This may be caused by heat acclimation or heat shock proteins produced in pests during lengthy exposures to nonlethal temperatures (Thomas and Shellie 2000). Based on the estimated heating rate of chestnuts achieved by RF energy (Hou et al. 2014), a heating rate of 5°C/min was used to determine the thermal death kinetics of fifth-instar *C. punctiferalis*.

Thermal Death Kinetics. The average survival rate for unheated fifth-instar *C. punctiferalis* was $97.49 \pm 0.13\%$, showing that the effect of handling on the final insect mortality was negligible. Consequently, the treatment mortality in subsequent temperature–time combination tests was not corrected by the control mortality. Table 2 shows coefficients of determination (R^2) for different reaction orders modeling the mortality response of *C. punctiferalis* to four treatment temperatures. Because the 0th-order model had the largest average coefficient of determination ($R^2 = 0.973$) over the four temperatures, it was selected for further model calculations. This contrasts with earlier studies

Table 1. Corrected mortality of *C. punctiferalis* at different life stages after heating at 5°C/min in HBS

Temperature + holding time	Eggs	Third-instars	Fifth-instars	Pupae
46°C + 8 min	100 ± 0a*	94.67 ± 5.03a	62.63 ± 1.59c	79.03 ± 6.02b
48°C + 4 min	94.02 ± 5.28a	86.20 ± 4.39b	59.43 ± 9.02c	61.43 ± 6.71c
50°C + 2 min	100 ± 0a	100 ± 0a	85.84 ± 4.44b	88.20 ± 5.19b

*Different letters in rows indicate that means are significantly different for different life stages ($P < 0.05$).

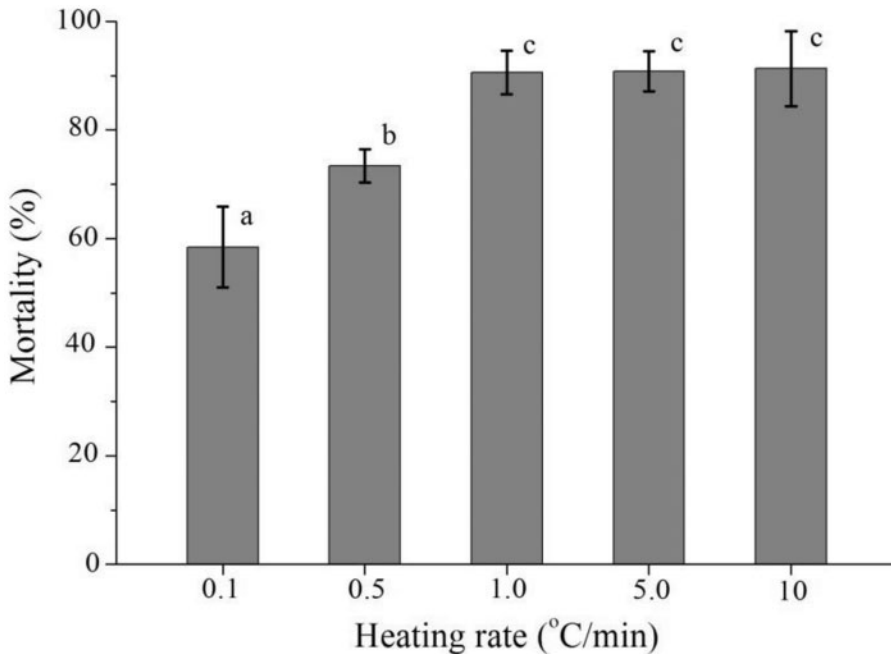


Fig. 1. The mortality of fifth-instar *C. punctiferalis* at 46°C for 10 min as influenced by heating rates. Different letters indicate that means are significantly different ($P < 0.05$).

on codling moth (Wang et al. 2002a), Indianmeal moth (Johnson et al. 2003), navel orangeworm (Wang et al. 2002b), and red flour beetle (Johnson et al. 2004), which followed 0.5th-order reaction model. This reaction model agreed with that for rice weevil (Yan et al. 2014).

The thermal mortality curves of fifth-instar *C. punctiferalis* with best-fit 0th-order model are shown in Figure 2. The slopes of the thermal mortality curves decreased several-fold when the treatment temperature increased from 44 to 50°C. The minimum holding time to achieve 100% mortality of 150 insects was ~55, 12, 6, and 3 min for 44, 46, 48, and 50°C, respectively.

Table 3 shows the model constants fitted by the 0th-order reaction model for thermal mortality of *C. punctiferalis*. As expected, the *k* value increased with increasing temperature, suggesting that higher temperatures require shorter exposures to achieve the same insect mortality. Table 4 shows the minimum time for 100% mortality of 150 insects and predicted LT to obtain 95, 99, 99.33, and 99.99% mortality.

Table 2. Determination of the best kinetic order (*n*) for thermal kill of fifth-instar *C. punctiferalis* at four temperatures by comparing the coefficients of determination (*R*²)

Temp. (°C)	<i>N</i> ₀	<i>n</i> = 0	<i>n</i> = 0.5	<i>n</i> = 1.0	<i>n</i> = 1.5	<i>n</i> = 2.0
44	150	0.975	0.889	0.732	0.602	0.537
46	150	0.973	0.911	0.817	0.715	0.632
48	150	0.983	0.907	0.778	0.695	0.668
50	150	0.962	0.979	0.865	0.78	0.677
Average	150	0.973	0.922	0.798	0.698	0.629

*N*₀ = the initial number of insects tested.

TDT Curve and Activation Energy. The TDT curve for fifth-instar *C. punctiferalis* is shown in Figure 3. The curve for fifth-instar *C. punctiferalis* was described by the linear regression equation $\log t = -0.214T + 11.08$ with the coefficient of determination *R*² = 0.964, where *t* is exposure time and *T* is treatment temperature. The *z* value obtained from the negative inverse of the slope of the TDT curve was 4.07°C, resulting in thermal death activation energy of 482.15 kJ/mol.

Figure 4 shows the Arrhenius plot for temperature effects on thermal death rates of fifth-instar *C. punctiferalis*. The regression equation was $\log k = -23.96 \times 1/T \times 1000 + 73.58$ with the coefficient of determination *R*² = 0.952. From the slope of the regression equation, the activation energy calculated by equation 5 was 481.53 kJ/mol, which was similar to that estimated by equation 4. This activation energy of *C. punctiferalis* was smaller than that for navel orangeworm (Wang et al. 2002b), Indianmeal moth (Johnson et al. 2003), and rice weevil (Yan et al. 2014), but slightly larger than that for codling moth (Wang et al. 2002a), suggesting that *C. punctiferalis* is less sensitive

Table 3. Thermal death constants of 0th-order reaction model for *C. punctiferalis* at four different temperatures

Temp. (°C)	$(N/N_0)^{1.0} = -kt + c$	
	<i>k</i>	<i>c</i>
44	0.016	0.878
46	0.098	1.132
48	0.176	1.096
50	0.475	1.190

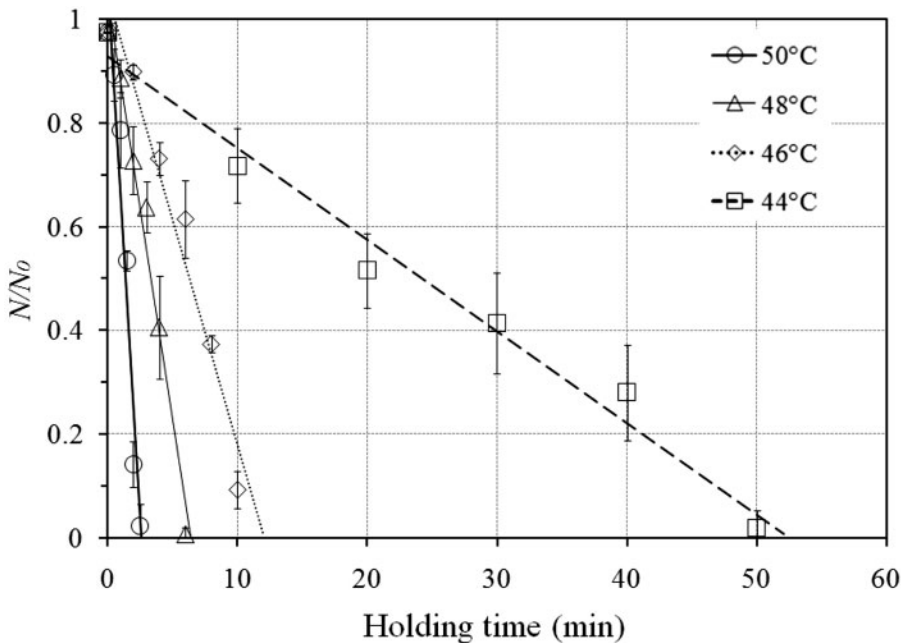


Fig. 2. Thermal mortality curves of fifth-instar *C. punctiferalis* at different temperatures and exposures. *N* and *N*₀ are the surviving and initial numbers of *C. punctiferalis*, respectively.

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

Temp. (°C)	N_0	Min. time for 100% mortality of 150 insects	Predicted treatment time (min) (95% CI)			
			LT ₉₅	LT ₉₉	LT _{99.33}	LT _{99.99}
44	150	55	51.75 (43.1–57.5)	54.25 (44.9–60.5)	54.47 (45.0–60.8)	54.87 (45.3–61.2)
46	150	12	11.04 (9.2–12.6)	11.45 (9.5–13.1)	11.48 (9.5–13.1)	11.55 (9.5–13.2)
48	150	6	5.94 (5.1–6.7)	6.17 (5.3–6.9)	6.19 (5.3–6.9)	6.23 (5.4–7.0)
50	150	3	2.40 (2.0–2.8)	2.48 (2.0–2.9)	2.49 (2.0–2.9)	2.51 (2.0–2.9)

N_0 = the initial number of insects tested.

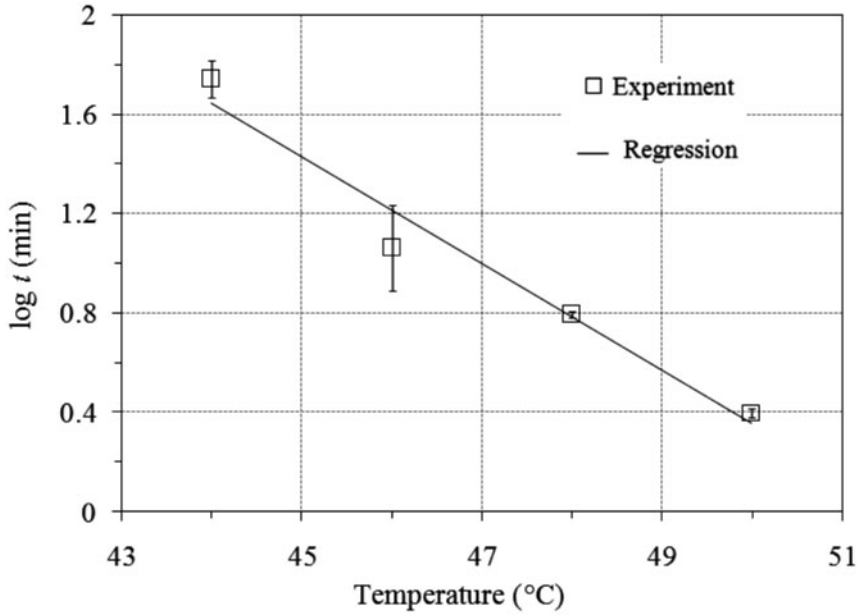


Fig. 3. Thermal mortality curve for fifth-instar *C. punctiferalis* at a heating rate of 5°C/min.

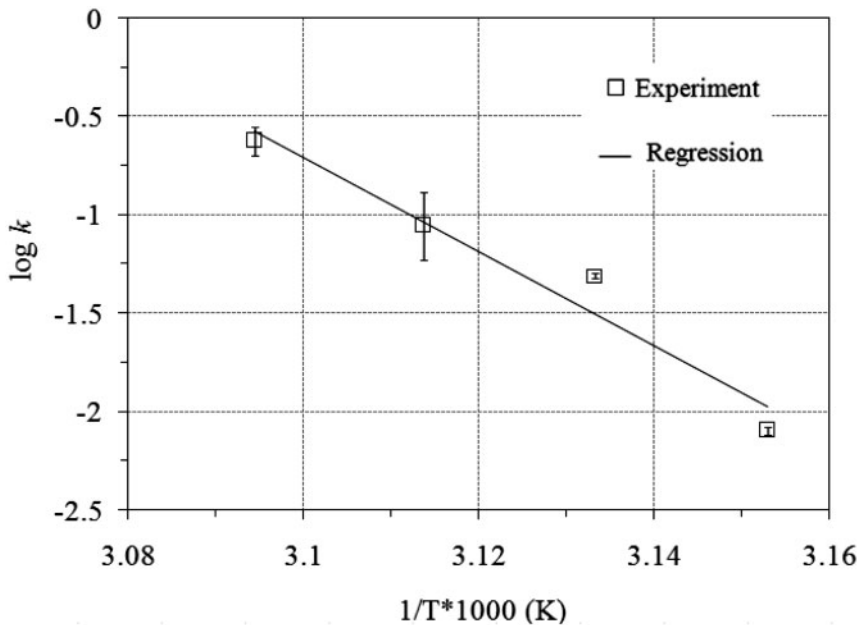


Fig. 4. Arrhenius plot for temperature effects on thermal death rates of fifth-instar *C. punctiferalis*.

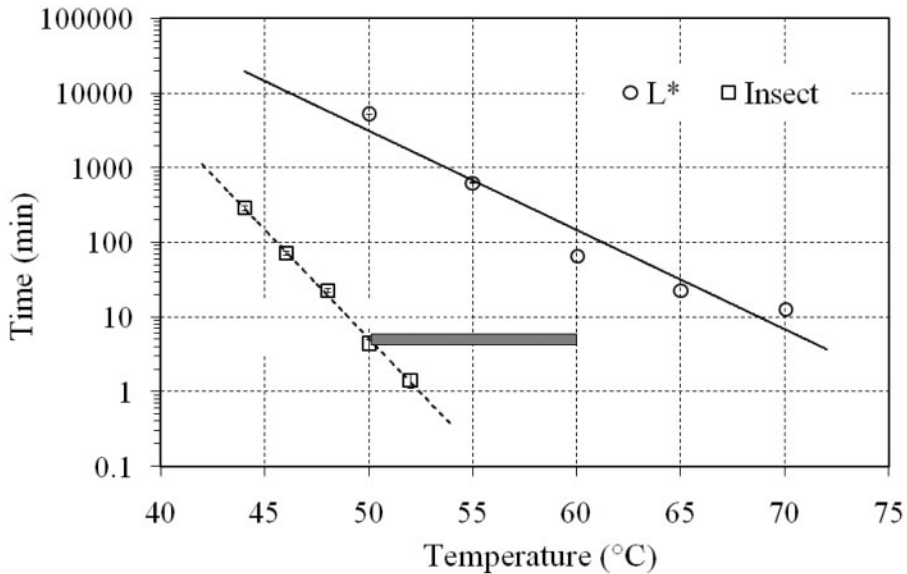


Fig. 5. Possible thermal treatment protocol region and proposed operating zone (gray zone) between product quality ($L^* = 50$) curves of chestnuts (Hou et al. 2015) and thermal death–time curves of *C. punctiferalis* to achieve 100% mortality.

to temperature changes than navel orangeworm, Indianmeal moth, and rice weevil, but more sensitive than codling moth.

Treatment Protocol to Control *C. punctiferalis* in Chestnuts. As the color of chestnuts is sensitive to heat, chestnut kernels become darkened or brown during thermal treatments, which is mainly quantified by the lightness measurement (L^*). Generally, L^* levels < 50 are unacceptable by consumers (Gamlath 2008, Nunes et al. 2013). By combining the temperature–time requirement for complete kill of *C. punctiferalis* with acceptable L^* values (≥ 50) for chestnut kernels during thermal treatment (Hou et al. 2015), a practical and effective treatment protocol could be defined (Fig. 5). A similar method was used for the development of thermal treatments to control codling moth in stone fruit and nuts (Yokoyama et al. 1991, Wang et al. 2008). Possible treatments include low temperatures with long exposures and high temperature with short exposures. Because of the need for high product throughputs and tolerance for large temperature variations, a thermal treatment protocol (grey zone in Fig. 5) of 5-min exposure to 50–60°C would be more practical, and could be used for disinfesting chestnuts without damaging chestnut color. This protocol could be successfully achieved by RF heating in chestnuts, as reported by Hou et al. (2014).

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