Tolerance of *Sitophilus zeamais* (Coleoptera: Curculionidae) to heated controlled atmosphere treatments

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ABSTRACT

Combination heat and controlled atmosphere (CA) postharvest phytosanitary treatments are environmentally friendly alternatives to chemical fumigants. A controlled atmosphere/heating block system (CA-HBS) was used to rapidly assess tolerances of adult maize weevil, *Sitophilus zeamais*, both under regular air (RA) and CA (1% O_2 and 15% CO_2) conditions. In the RA treatment, thermal death kinetics for *S. zeamais* adults were determined at temperatures between 46 °C and 52 °C at a heating rate of 5 °C/min. The results showed that thermal death curves of *S. zeamais* adults followed a 0th-order kinetic reaction model. The required holding times for achieving 100% mortality were 165, 40, 14, and 4 min at 46, 48, 50 and 52 °C, respectively. The activation energy for killing *S. zeamais* adults was 526.7 kJ/mol. The effects of CA at various temperature-time combinations and heating rates on insect mortality were evaluated. The mortality of *S. zeamais* adults was higher under CA treatment than in the heat treatment alone. The slowest heating rate (0.1 °C/min) achieved the highest insect mortality in CA treatments but lowest mortality in RA treatments. The information obtained from the CA-HBS can be used to develop combination heat and CA treatments against *S. zeamais*.

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1. Introduction

Grain is often infested with various stored-product insects, which cause severe economic losses (Moreno-Martinez et al., 2000). The maize weevil, *Sitophilus zeamais* (Motschulsky), is one of the major pests of stored grain (Carvalho et al., 2012; Fragoso et al., 2005). Chemical fumigations with methyl bromide (MeBr) have been widely used to control insect pests. There are increasing public concerns over the use of agricultural chemicals that are harmful to the environment and human health (Bulathsinghala and Shaw, 2014), and the Montreal Protocol has mandated phasing out the use and production of MeBr for postharvest phytosanitary purposes in developing countries by 2015 (USEPA, 2001). Non-chemical phytosanitary treatments are needed as alternatives to fumigants.

Several alternative non-chemical treatments have been suggested, including high temperature and controlled atmosphere (CA) treatments (Fleurat-Lessard and Dupuis, 2010; Sen et al., 2010). The mechanism of CA treatments relies on interference with insect respiration and metabolism, and is highly temperature dependent, with increasing temperatures reducing the exposure times needed for control (Bailey and Banks, 1980; Donahaye et al., 1996; Storey, 1975). Combining heat with CA has been shown to reduce the effect of treatments on product quality (Fleurat-Lessard, 1990; Johnson and Neven, 2010). Heat combined with CA treatment has been suggested to control insect pests in fresh fruits and stored products (Hansen et al., 2011; Neven, 2005; Neven and Rehfield-Ray, 2006; Shellie et al., 1997), and shows potential as an alternative to chemical fumigants.

Previous studies on combining high temperature and CA (Donahaye et al., 1996; Neven and Rehfield-Ray, 2006) used experimental protocols that were labor intensive, had low test efficiency, and had difficulty in maintaining temperature and gas concentration stability. A more stable laboratory system for quickly assessing the treatment tolerance of target insects is needed. Neven (2008) developed a controlled atmosphere/water bath (CA-WB) system to simulate the slow heating rates found in treatment systems for fresh fruit such as CATTS (Controlled Atmosphere Temperature Treatment System, Neven and Mitcham, 1996). Johnson

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and Neven (2010) found that the CA-WB system could be used as a more efficient laboratory alternative to CATTS for testing *Thaumatotibia leucotreta* (Meyrick). But this system is relatively slow, labor-intensive, and not able to treat large numbers of insects at one time (Neven et al., 2012). Recently, Neven et al. (2012) and Li et al. (2015) developed a more reliable device for heat-CA treatments based on a unique experimental heating block system (HBS). The controlled atmosphere/heating block system (CA-HBS) uses a programmable heating block to which controlled gases are added, producing a wide range of heating rates, temperatures and CA concentrations. The CA-HBS could be used as an effective device to evaluate *S. zeamais* mortality under combined heat and CA conditions.

The previous HBS was developed for testing responses of insects to high temperatures and rapid heating rates (Ikediala et al., 2000), and modified to allow for the addition of CA to the test chamber (Neven et al., 2012; Wang et al., 2002b). The HBS has been widely used to obtain thermal death kinetic data in ambient air for several insect pests of fruits and nuts (Gazit et al., 2004; Johnson et al., 2003; Wang et al., 2002a, 2002b). Based on the data the 0.5th-order kinetic model has been chosen for codling moth, *Cydia pomonella* (L.) (Wang et al., 2002a), *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), and Mexican fruit fly, *Anastrepha ludens* (Loew) (Hallman et al., 2005) together with 0th-order kinetic model for rice weevil, *Sitophilus oryzae* (L.) (Yan et al., 2014). Thermal death kinetics for insect pests are critical in developing effective treatment protocols for combined heat and CA.

Heating rate has been shown to have a significant effect on thermal mortality of insects in ambient air. Using relatively slow heating rates (0.067–0.2 °C/min), Neven (1998) and Thomas and Shellie (2000) reported that slower heating rates required longer exposures at the treatment temperature to achieve the same mortality as more rapid heating rates. These differences in insect mortality are most likely the result of acclimation during the slower heating rate, most probably through the production of heat shock proteins (Thomas and Shellie, 2000). Wang et al. (2005) observed that at heating rates of 1 °C/min or faster test insects were unable to acclimate to the treatment. Yan et al. (2014) found similar mortality at heating rates of 1 °C/min, 5 °C/min and 10 °C/min, but reduced insect mortality at heating rates of 0.1 °C/min and 0.5 °C/min, suggesting that rapid heating should be used in the development of effective postharvest thermal treatment protocols. Johnson and Neven (2010) reported that a substantially longer time was required to achieve the desired mortality of eggs and larval stages of *T. leucotreta* at the slower heating rate (12 °C/h) in heated CA treatments. Therefore, understanding the effect of different heating rates on insect mortality is needed in developing an effective heat and CA treatment protocol for *S. zeamais*.

Objectives of this study were to 1) develop thermal death kinetics for *S. zeamais* under RA, 2) determine the effect of heated CA treatments on the *S. zeamais* mortality and compare with heat alone, and 3) explore the effect of heating rates on mortality of insects in the heated CA treatments.

2. Materials and methods

2.1. CA-HBS

Treatments were done using a controlled atmosphere/heating block system (CA-HBS) (Fig. 1). The CA-HBS was composed of three gas cylinders, a gas mixing flask, an O_2/CO_2 gas analyzer (CYCK-201, Yantai Venture Control Engineering Co. Ltd., Yantai, China), a pair of heating blocks (HBS), and a computer. The CA-HBS provides

relatively stable gas composition, negligible leakage rates and uniform temperature distributions (Li et al., 2015). Variation of O_2 and CO_2 concentration was $\leq 0.016\%$ and 0.059%, respectively. Because gas was preheated in channels running through the top and bottom blocks before entering the treatment chamber, block temperature differences from the set-point was <0.5 °C (Li et al., 2015). Detailed descriptions of the HBS can be found in Ikediala et al. (2000), Wang et al. (2002b), Johnson et al. (2003), and Yin et al. (2006).

2.2. Insects

The HBS system is more difficult to use with internal stages such as *S. zeamais* larvae and pupae. Because removal of internal stages from the seeds causes high mortality, they must be treated within the seed. Insulation by the seed slows the heating rate and makes it difficult to quantify. The treatment response of internal stages is normally measured by adult emergence, which complicates direct comparisons between stages. The heating rates for treating external, mobile adult stages are easier to quantify, and evaluation is immediate and consistent. For these reasons, we selected the adult stage for this initial study (Yan et al., 2014). Test insects used in this study were from a laboratory culture originally obtained from Yangling, Shaanxi, China, and were reared on insecticide-free wheat held in 600 mL glass jars under ambient conditions of about 26 °C with 65% relative humidity and a photoperiod of 14:10 (L:D) h using artificial light.

2.3. Treatments

To determine the effect of heat alone and develop thermal mortality curves for *S. zeamais*, treatments were first conducted under regular room air (RA) conditions. In these treatments, there was no air running through the heating block. Based on results from thermal death trials for *T. castaneum* (Johnson et al., 2004) and *S. oryzae* (Yan et al., 2014), four treatment temperatures (46, 48, 50 and 52 °C) and four or five exposure times (0.5–150 min) were selected to obtain a wide range of insect mortality, up to 100%, of adult *S. zeamais*.

Based on results from heated CA treatments for *T. leucotreta* (Johnson and Neven, 2010) and the oriental fruit moth (Neven et al., 2012), CA gas concentrations of 1% O_2 and 15% CO_2 was chosen to compare insect mortality response under heated CA and heated RA. The same CA concentration was used to explore the effect of heating rates on insect mortality under heated CA treatments. A starting temperature of 26 °C and heating rate of 5 °C/min were used for all treatments. The gas flow rate through the CA-HBS was between 460 and 490 mL/min. To test the effect of heated CA treatments on adult *S. zeamais*, insects were exposed to 44 °C for 60, 90, and 120 min, 46 °C for 30, 60, and 90 min and 48 °C for 10, 20, and 30 min. Gas was released from the cylinders and premixed to target levels (Fig. 1). The O_2/CO_2 analyzer was used to determine the concentration of the gas as it entered the heating block. Once the gas concentration in the insect chamber reached the desired level and was stable, the top heating block was removed, pre-counted test insects were placed on the bottom heating block and the top heating block was immediately replaced. The heat treatment began after the gas level in the system re-equilibrated at the set-point level, usually within 6 min after the addition of test insects (Li et al., 2015).

At the beginning of each treatment, approximately 50 actively moving adult *S. zeamais* were randomly selected and placed in a nylon-mesh bag to limit their movement (Yan et al., 2014) and the bag was placed in the treatment chamber. At the end of each treatment, the nylon-mesh bag was immediately removed from the

block. Test insects were then removed from the bags and placed in 200 mL glass jars containing 10 g wheat. Adults were evaluated 6 days later and considered to be dead if no movement was observed. Control insects were placed in the unheated treatment chamber for the longest exposure time of the study (150 min).

Based on previous test results, a treatment temperature-time combination of 48 °C for 30 min was selected to compare the effect of heating rates on insect mortality. Heating rates of 0.1, 0.5, 1, 5 and 10 °C/min were compared under both RA and CA environment (1% O_2 / 15% CO_2). The slower heating rates ($\leq$1 °C/min) were selected to simulate hot air and hot water heat treatments, and the faster heating rates ($\geq$5 °C/min) were used to simulate radio frequency (RF) and microwave treatments.

All treatments were replicated three times and mean values and standard deviations were calculated. The mean values of the insect mortality at different heating rates under 1% O_2 / 15% CO_2 CA environment were separated at $P = 0.05$ level using least significant difference (LSD) t-test.

2.4. Kinetic modeling of insect thermal mortality response

A fundamental kinetic model has been used to describe the mortality response to heat in regular air of various insect species, including *A. transitella* (Wang et al., 2002b), *C. pomonella* (Wang et al., 2002a), *P. interpunctella* (Johnson et al., 2003), *T. castaneum* (Johnson et al., 2004) and *S. oryzae* (Yan et al., 2014). The kinetic model is described as follows:

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

where n is the kinetic order of reaction; k is the thermal death rate constant; t is the exposure time (min); N and N_0 are the surviving and initial numbers of *S. zeamais*. The integration form of Equation (1) can be converted to different reaction orders as follows:

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

Equation (2) was used to analyze survival (N/N_0) versus time using 0, 0.5, 1, 1.5 and 2 reaction orders. The most suitable order was selected by comparing the average coefficients of determination (R^2) for the regressions at the four treatment temperatures. The constants k and c of the best-fitted regression equation were obtained and the model was used to estimate the exposure times needed to obtain 90, 95, 99.33 and 99.99% mortality (LT_{90}, LT_{95}, $LT_{99.33}$ and $LT_{99.99}$, respectively). The thermal death time (TDT) curve of adult *S. zeamais* was developed by plotting the minimum exposure time required to achieve 100% mortality against temperature on a semi-log scale (Wang et al., 2002a). The z value (°C), defined as the temperature difference by which the mortality rate is altered by a factor of 10, was calculated from the negative inverse of the slope of the TDT curve.

2.5. Activation energy

The thermal death activation energy (E_a, J/mol) can be used to determine the sensitivity of insects to changes in temperature. According to the methods used in Hallman et al. (2005) and Yan et al. (2014), E_a of *S. zeamais* adults was calculated by the following two equations:

$$E_a = \frac{2.303RT_{min}T_{max}}{z} \tag{3}$$

where R is the universal gas constant (8.314 J/mol K), and T_{min} and T_{max} are the minimum and maximum absolute temperatures (K) of the test range, respectively. The second equation is based on an Arrhenius plot as follows:

$$log k = log k_0 - \frac{E_a}{2.303RT} \tag{4}$$

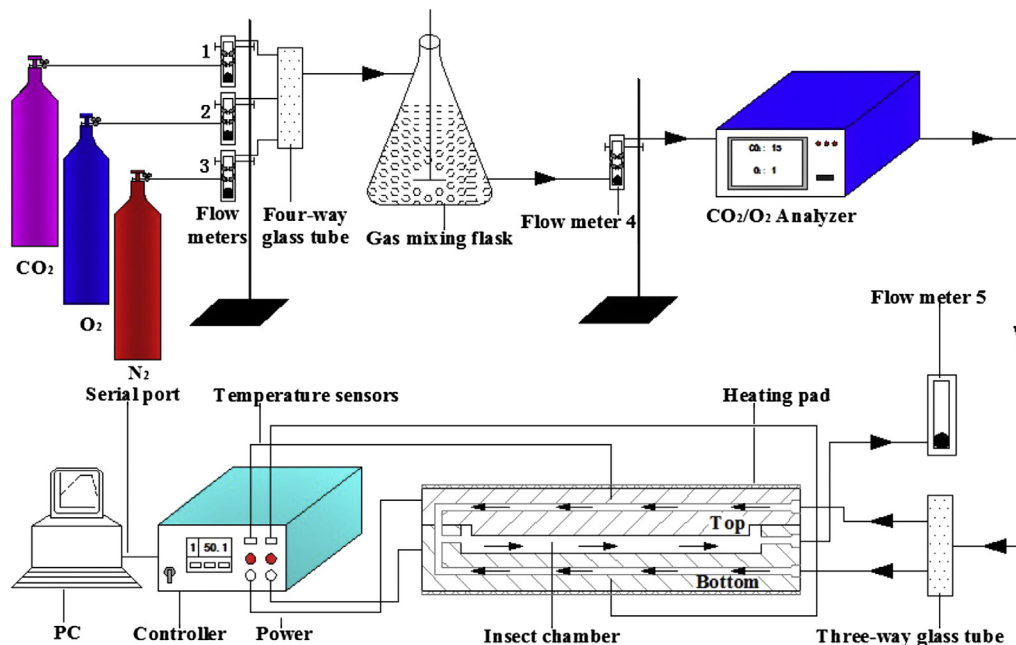where k_0 is the reference thermal death rate constant (min^{-1}).

Fig. 1. Diagram of controlled atmosphere/heating block system, including gas cylinders, flow meters, gas mixing flask, O_2/CO_2 analyzer, and heating blocks with computer control and monitoring (Li et al., 2015).

3. Results and discussion

3.1. Thermal death kinetics of adult Sitophilus zeamais under regular air

The survival rate of untreated controls was high (95.0 ± 1.4%), suggesting that handling had little effect on test insects. Therefore, treatment mortality data were not corrected for control mortality. Table 1 lists coefficients of determination (R^2) derived for each reaction order and treatment temperature. As with *S. oryzae* adults (Yan et al., 2014), the 0th order reaction model produced the highest average R^2 value. Consequently, it was selected for further calculations. Thermal mortality curves for adult *S. zeamais* are presented in Fig. 2.

Table 2 shows the thermal death constants for the 0th-order reaction model. The thermal death rate constant k increased with temperature in agreement with earlier studies (Wang et al., 2002b; Yan et al., 2014). In addition, the c value was within the margin of error for the ideal of 1 at time zero for all four temperatures. Table 3 shows that the minimum time to reach a complete kill of 150 insects and the predicted LT_{90}, LT_{95}, $LT_{99.33}$ and $LT_{99.99}$ by the established thermal death kinetic model. The predicted values for $LT_{99.33}$ (1 survivor out of 150 test insects) were close to the observed minimum time for 100% mortality. The predicted lethal times to obtain 99.99% mortality for *S. zeamais* adults was much higher than those for *S. oryzae* adults (Yan et al., 2014), with *S. zeamais* adults requiring treatment temperatures of 2 °C more to obtain comparable LT values. These results also indicated that *S. zeamais* adults were less tolerant than red flour beetle larvae at 48 °C but more tolerant at 50 and 52 °C (Johnson et al., 2004).

Temperatures of 46 and 48 °C produced lengthy $LT_{99.99}$ values for *S. zeamais* adults of 164.6 and 42.5 min, respectively, suggesting these temperatures would not provide rapid control of adults. More practical treatment times were obtained with higher temperatures, with an $LT_{99.99}$ of 4 min at 52 °C. Such rapid treatment times are obtained easily using radio frequency energy, and may also avoid degradation of product quality (Wang et al., 2002c). Although the most thermotolerant stage of *S. zeamais* has not been determined using the HBS, Adler (2008) found that *S. zeamais* adults were the most tolerant stage at 50 and 55 °C. This suggests that the estimated $LT_{99.99}$ exposure of 4 min at 52 °C may be a suitable treatment protocol for *S. zeamais*.

3.2. Thermal death time curve and activation energy

Fig. 3 shows the thermal death time (TDT) curve for adult *S. zeamais* at a heating rate of 5 °C/min. The curve was described by the linear regression equation $\log t = 14.382 - 0.2651T$ with $R^2 = 0.997$. The z value obtained from the TDT curve was 3.8 °C, and the thermal death activation energy (E_a) was estimated to be 526.7 kJ/mol using Eq. (3).

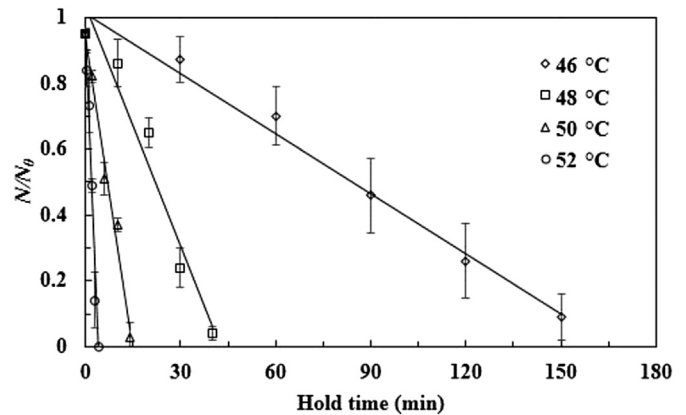The Arrhenius plot for temperature effects on thermal death

Fig. 2. Thermal mortality curves of adult *Sitophilus zeamais* at four different temperatures under regular air.

rates of adult *S. zeamais*, (Fig. 4) was described by the linear regression equation $\log k = 82.868 - 27.245 \times 1/T*1000$ with $R^2 = 0.995$. An activation energy of 521.7 kJ/mol was obtained using Eq. (4), similar to that obtained by the TDT curve using Eq. (3).

Increased activation energy indicates an increased sensitivity to changes in temperature. The E_a values of adult *S. zeamais* obtained from the TDT curve and Arrhenius plot were similar to that of *A. transitella* (519.0 kJ/mol) (Wang et al., 2002b), adult *S. oryzae* (505.0 kJ/mol) (Yan et al., 2014), and *P. interpunctella* (506.3 kJ/mol) (Johnson et al., 2003), higher than that of *C. pomonella* (472.0 kJ/mol) (Wang et al., 2002a), but smaller than that of *T. castaneum* (814.1 kJ/mol) (Johnson et al., 2004). This suggests that the response of *S. zeamais* to changes in temperature would be similar to most of the pest species studied to date.

3.3. Heated controlled atmosphere (CA) treatments

Percent mortalities of adult *S. zeamais* after heated CA treatments were compared with those obtained using heat treatment alone (Fig. 5). In all treatments, mortality was higher in heated CA than under heat treatment, with a sharp increase in mortality in the heated CA treatments as exposure increased. At 44 °C, 96.0% ± 2.0% mortality was obtained after a 2 h exposure to CA compared to 33.5 ± 7.8% mortality under RA. For each temperature, the addition of CA reduced the treatment time needed to achieve the desired mortality. Similar results have been reported in the literature. Fourth-instar oriental fruit moths were found to be more sensitive to CA than RA heat treatment (Neven et al., 2012). Reducing the O_2 concentration from 21% to 1% during a 44 °C heat treatment corresponded to a reduced exposure time from 5 h to 3.5 h to achieve 100% larval mortality (Shellie et al., 1997). Lurie et al. (2004) also reported that shorter exposures provided control under 0.05% O_2 as

Table 1
Coefficients of determination (R^2) from kinetic order (n) models for thermal mortality of adult *Sitophilus zeamais* at four temperatures.

Temperature (°C)	R^2 for different order n				
	$n = 0$	$n = 0.5$	$n = 1$	$n = 1.5$	$n = 2$
46	0.985	0.951	0.881	0.770	0.661
48	0.957	0.912	0.824	0.699	0.602
50	0.987	0.930	0.797	0.668	0.596
52	0.989	0.952	0.746	0.556	0.517
Mean	0.979	0.936	0.812	0.673	0.594

Table 2
The 0th-order reaction model constants for adult *Sitophilus zeamais* at four temperatures.

Temperature (°C)	Parameters for $(N/N_0)^{1-0} = -kt + c$	
	k	c
46	0.0061	1.0100
48	0.0244	1.0360
50	0.0635	0.9424
52	0.2495	0.9616

Table 3
Comparison of lethal times (min) obtained by experiments and 0th-order kinetic model for adult *Sitophilus zeamais* at four temperatures.

Temp. (°C)	Minimum time for 100% mortality of 150 insects	Predicted treatment time (min) (95% CI)			
		LT_{90}	LT_{95}	$LT_{99.33}$	$LT_{99.99}$
46	165	149.18 (138.99—158.67)	157.38 (146.38—167.51)	164.48 (152.75—175.19)	165.58 (153.72—176.37)
48	40	38.36 (28.83—46.30)	40.41 (30.18—48.88)	42.18 (31.33—51.12)	42.45 (31.50—51.47)
50	14	13.27 (11.36—14.99)	14.05 (12.00—15.90)	14.74 (12.55—16.69)	14.84 (12.64—16.81)
52	4	3.45 (3.12—3.75)	3.65 (3.30—3.97)	3.83 (3.45—4.16)	3.85 (3.47—4.19)

compared to ambient air at the same temperature. Carvalho et al. (2012) confirmed that increasing temperature decreased the treatment time needed to control *Sitophilus* spp. under CA.

3.4. Effect of heating rates on insect mortality

Fig. 6 shows the effect of five heating rates on insect mortality at 48 °C for 30 min under RA and CA conditions. The time from the initial (26 °C) to the final temperature (48 °C) was 220, 44, 22, 4.4, and 2.2 min when the heating rates were 0.1, 0.5, 1, 5, and 10 °C/min, respectively. For RA treatments (Fig. 6a), the results suggest that higher heating rates achieve higher mortality, and are similar

to results in Yan et al. (2014). In CA treatments (Fig. 6b), however, there was no significant difference ($P > 0.05$) in average insect mortality when heating rate was $\geq$0.5 °C/min. Mortality at the slowest heating rate (0.1 °C/min) was significantly higher ($P < 0.05$) than at the faster heating rates ($\geq$0.5 °C/min). The increased mortality in the CA-HBS treatments using the slow heating rate of 0.1 °C/min may be caused by the lengthy exposure to CA during the extended ramp period. These findings differ from those observed in earlier studies using heated CA against *T. leucotreta* (Johnson and Neven, 2010), in which the faster heating rate (0.4 °C/min) reduced effective treatment time needed for control treatments when compared to the slower heating rate (0.2 °C/min).

4. Conclusions

Mortality of *Sitophilus zeamais* was influenced by a variety of different treatment conditions, including temperature-time combinations, controlled atmosphere environment and heating rates. The fundamental 0th reaction model described the response of adult *S. zeamais* to regular air heat treatment and may be useful in developing heat treatment protocols for *S. zeamais*. The controlled atmosphere/heating block system was useful in rapidly assessing tolerance of *S. zeamais* to heated CA treatments. The mortality of adult *S. zeamais* under the CA treatment was significantly higher than that under the RA treatment. The slowest heating rate (0.1 °C/min) achieved the highest mortality under the CA treatment but lowest mortality under the RA treatment. Heated controlled atmosphere treatments have potential to replace chemical fumigation for control of insect pests.
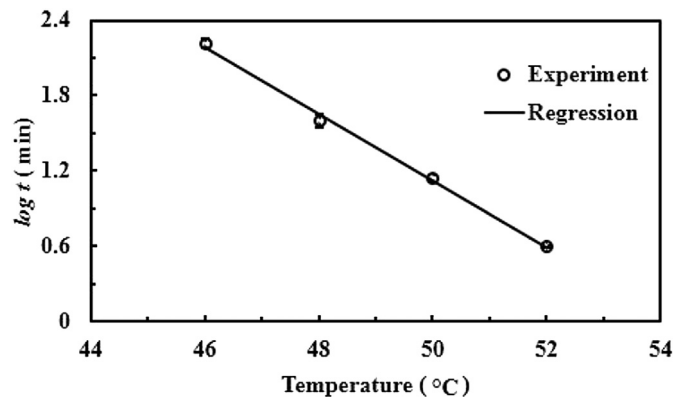

Fig. 3. Thermal death time curve for adult *Sitophilus zeamais* at a heating rate of 5 °C/min. Line represents linear regression $\log t = 14.382 - 0.2651T$ ($R^2 = 0.997$), where t is exposure time (min) and T is treatment temperature (°C).
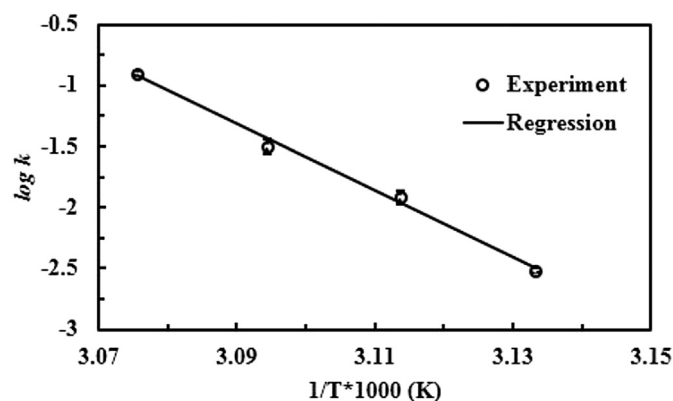

Fig. 4. Arrhenius plot for temperature effects on thermal death rates of *Sitophilus zeamais*. Line represents linear regression $\log k = 82.868 - 27.245 \times 1/T \times 1000$ ($R^2 = 0.995$) where k is the thermal death rate constant (min^{-1}) and T is the treatment temperature (K).
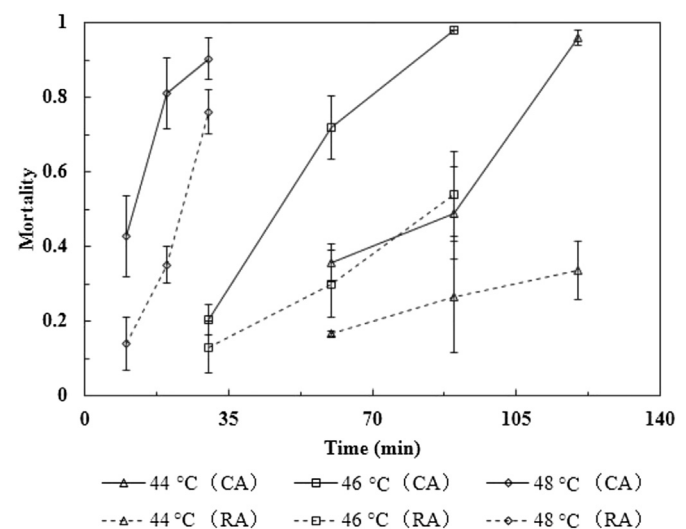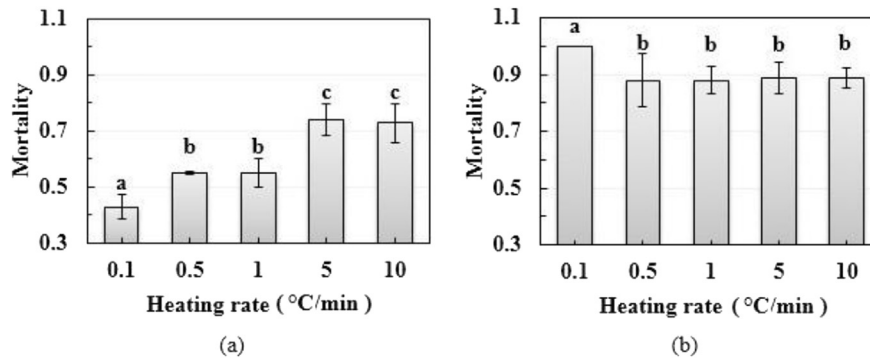

Fig. 5. Comparison of mean ($\pm$SD) mortality (%) of *Sitophilus zeamais* after regular air (RA) and controlled atmosphere (CA, 1% O_2/15% CO_2) heat treatments at three temperatures and times with a heating rate of 5 °C/min.

Fig. 6. The mortality of *S. zeamais* after heat treatments (48 °C for 30 min) at different heating rates under (a) RA and (b) CA (1% O_2/15% CO_2). Different letters indicate that means were significantly different ($P < 0.05$).

Acknowledgments

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