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Research Paper

Performance of controlled atmosphere/heating block systems for assessing insect thermotolerance

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Heated controlled atmosphere (CA) treatments have potential as alternatives to chemical fumigation for disinfesting postharvest fresh and stored products. To determine accurately the minimal thermal requirements to kill target insects over a wide range of temperatures and CA conditions, it is desirable to develop a model system to assess quickly the target insect thermotolerance. This study evaluated the gas tightness of the new controlled atmosphere/heating block system (CA–HBS) and the stability of gas concentrations, and determined temperature variations in the treatment chamber with and without added gas and under different gas channel designs and heating rates. The results showed that the new CA–HBS had a relatively constant leakage rate and kept O_2 and CO_2 concentration variations to within ±0.067% and ±0.167% at three set points (1% O_2:15% CO_2, 2% O_2:17% CO_2, and 2% O_2:20% CO_2), resulting in relatively stable gas compositions. With the long gas channel design, temperature variations in the treatment chamber were not influenced by the addition of gas or by heating rates. The performance of the CA–HBS indicated that this model system could be used for rapid assessment of pest thermotolerance.

1. Introduction

Postharvest products are of ten infested by various storage pests, resulting in more than 20% losses in developing countries (Doumbia et al., 2014). Chemical fumigations with methyl bromide have been widely used to control insects in stored products. Increasing public concerns over the use of agricultural chemicals that are harmful to the environment and human health (Bulathsinghala & Shaw, 2014), have increased the need to reduce their use, and the Montreal Protocol has mandated phasing out the use and production of methyl bromide for postharvest phytosanitary purposes by 2015 in developing countries (USEPA, 2001). Therefore, it is necessary to develop an alternative non-chemical treatment for postharvest disinfestation of stored products.

Several alternative non-chemical treatments have been suggested, including low pressure (Kucerova, Kyhos, Aulicky, & Stejskal, 2013), cold storage (Nakakita & Ikenaga, 1997), irradiation (Follett et al., 2013), controlled atmosphere (CA,

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Carvalho et al., 2012; Conyers & Bell, 2007), radio frequency (Wang, Monzon, Johnson, Mitcham, & Tang, 2007b) and microwave (Vadivambal, Jayas, & White, 2008). Low pressure, cold storage and CA require lengthy treatment times for disinfestation, which may also cause loss of product quality. Irradiation is effective for postharvest pest control in many commodities, but few dedicated irradiation facilities are available and a number of export markets (Japan, Taiwan, and the EU) severely limit or ban irradiated products (Follett & Weinert, 2012). Although radio frequency and microwave treatments may result in non-uniform heating, they show promise for disinfestation of low moisture products (Wang, Zhang, Gao, Tang, & Wang, 2014). The mechanism of CA treatments relies on interference with insect respiration and metabolism, and is highly temperature dependent (Donahaye, Navarro, Rindner, & Azrieli, 1996; Navarro, 2006). Combining rapid heating with CA could reduce the exposure times necessary for insect mortality without adversely affecting product quality (Fleurat-Lessard, 1990; Neven, Wang, & Tang, 2012; Sen, Meyvaci, Turanli, & Aksoy, 2010; Soderstrom, Brandl, & Mackey, 1996). Therefore, it is important to obtain accurate information on the minimal requirements for mortality of target insects over a wide range of temperatures and CA conditions to allow flexibility for treatment development.

Thermal response studies and efficacy tests are necessary steps to develop effective disinfestation treatment protocols, but they are time consuming and costly. A model system is needed to quickly assess the thermal response and determine the most thermotolerant life stage of target insects (Neven et al., 2012). For example, a unique experimental heating block system (HBS) has been developed for testing responses of insects to high temperatures and heating rates (Ikediala, Tang, & Wig, 2000; Johnson, Wang, & Tang, 2003; Wang, Ikediala, Tang, & Hansen, 2002; Wang, Tang, Johnson, & Hansen, 2002). The HBS is more accurate and versatile than previously reported methods for studying thermal mortality of insects. The HBS can be programmed to simulate the heating rate of the interior of products when subjected to different heating methods, such as hot air, hot water and radio frequency (RF) treatments. This method eliminates the effect of heat transfer in various hot air/water experiments on the intrinsic thermal death kinetics of insect pests. The HBS has generated highly repeatable results in ambient air for codling moth, navel orange worm, Indian meal moth, Mediterranean fruit fly, and Mexican fruit fly (Gazit, Rossler, Wang, Tang, & Lurie, 2004; Hallman, Wang, & Tang, 2005; Johnson et al., 2003; Wang, Ikediala, et al., 2002; Wang, Tang, et al., 2002). Thermal mortality data developed with the HBS have been validated using nuts or fresh fruits infested with target insect pests, including apples (Wang, Birla, Tang, & Hansen, 2006), cherries (Feng, Hansen, Biasi, Tang, & Mitcham, 2004; Hansen, Wang, & Tang, 2004), and walnuts (Mitcham et al., 2004; Wang, Monzon, Johnson, Mitcham, & Tang, 2007a, 2007b).

The use of commodity for initial dose response studies is not always practical, and often results in more variability than carefully controlled laboratory tests. Thus, the model systems are developed to quickly screen the most thermotolerant life stage of the target insects and determine the dosage required to achieve phytosanitary levels when subjected to CA and thermal treatments. For example, Donahaye et al. (1996) studied the effects of different gas concentrations on the mortality of *Tribolium castaneum* under temperature and modified atmospheres by laboratory experiments. But these previous systems were relatively slow, labour-intensive, and not amenable to treating large numbers of insects under accurate temperatures, heating rates and gas concentrations. Neven et al. (2012) modified the existing HBS to add controlled atmospheres and studied the effects of CA and heat on Oriental fruit moth mortality. The HBS has been used to test the response of storage pests to heat treatments (Johnson et al., 2003, 2004), which indicated that it can be suitable for developing an improvement system to test the response of storage pests to heated controlled atmosphere treatments. However, undetected variations in block temperatures and gas concentrations might be responsible for the observed variation in test insect mortality.

Stability of block temperatures, gas concentrations and heating rates are important performance characteristics of the new HBS, and changes to these parameters may affect test insect response (Das, Gurakan, & Bayindirli, 2006; Neven, 1998; Wang et al., 2006). In particular, stability of gas concentrations in the insect treatment chamber is an important performance characteristic for determining insect responses to CA (Chiappini, Molinari, & Cravedi, 2009; Donahaye et al., 1996; Moleyar & Narasimham, 1994). Heating rates have clear effects on insect thermal mortality (Neven, 1998; Thomas & Shellie, 2000; Yan, Huang, Zhu, Johnson, & Wang, 2014). The HBS can be used to simulate the slow heating rates in bulk stored products in conventional thermal treatments and fast heating rates in RF heating treatments (Wang et al., 2007a, 2007b). With the HBS, Wang, Ikediala, et al. (2002) studied the effect of heating rates on thermal death kinetics for codling moth in ambient air, and reported that the lethal time (LT) accumulated during the ramp period varied with the heating rate. As temperature and flow rate of added gases may affect heating rates and temperature uniformity, these parameters need to be determined before beginning insect thermal death kinetic tests.

The general objective of this research was to avoid the confounding effects of heat transfer for different sized products and provide basic heat treatment parameters that may be used in developing pest control treatments for a variety of treatment methods. Specific objectives were to 1) develop a model HBS suitable for studying pest thermotolerance under heated controlled atmosphere treatments, 2) determine the gas tightness of the modified CA–HBS, 3) evaluate the stability of target gas concentrations, 4) determine the block temperatures as influenced by gas channel designs and block heating rates in the CA conditions, and 5) apply the CA–HBS to assess the insect mortality.

2. Materials and methods

2.1. *Description of controlled atmosphere/heating block systems*

The controlled atmosphere/heating block system (CA–HBS) was composed of three gas cylinders, a gas mixing flask, an

O_2/CO_2 gas analyser, the heating block system (HBS), and a computer (Fig. 1). The three gas cylinders contained 40 l of CO_2, O_2 and N_2 obtained from a local gas factory in Yangling, Shaanxi, China. Gas valves were used to provide no more than 475.39 kPa (68.95 psi) total pressure for all three lines (Neven et al., 2012). Three flow meters (LZB-3WB, Changzhou Ruiming Instrument Factory, Changzhou, China) were used to control and monitor the flow rates of individual gases. Gases were mixed first through a four-way tube and then in a 5 l flask. For continuous and uniform mixing of the gases, a plastic stirring rod driven by an electric mixer (JJ-1, Changzhou Guohua Electric Appliance Co., Ltd., Changzhou, China) at about 200 rev min^{-1} was inserted into the flask. A fourth flow meter was used to control and monitor the gas flow rate into the heating block. The gas composition entering the heating block was monitored by O_2/CO_2 analyser (CYCK-201, Yantai Venture Control Engineering Co. Ltd., Yantai, China) with a detection range of 0–25%, resolution ratio of 0.1%, and sensitivity within ±2% for each gas. After leaving the gas analyser and depending upon the gas channel design being used, the gas was either fed directly into the treatment chamber through a channel in the bottom block, or it was split via a three-way glass tube and directed into gas channels in the top and bottom heating block. A fifth flow meter measured the speed of the gas as it exited the HBS. For studies on fresh fruit pests using the heat block system, filter paper soaked with nipagin solution or humidified air added through the gas channels could be used to increase humidity levels (Gazit et al., 2004; Neven et al., 2012). For storage insects from dry products, such as nuts and beans, the gas was not humidified in this system to simulate the similar dry environment (Johnson et al., 2003, 2004; Wang, Tang, et al., 2002).

The HBS was composed of top and bottom aluminium blocks (254 mm × 254 mm × 40 mm) to form an insect treatment chamber (214 mm × 214 mm × 6 mm). A rubber O-ring between the two blocks and petroleum jelly coated on the contact surface between the two blocks achieved a tight seal. The HBS was controlled by a data acquisition/control unit and the temperatures of the top and bottom blocks were measured by calibrated type-T thermocouples sensors. Two proportional-integral-derivative (PID) controllers (I32, Omega Engineering, Inc., Stamford, CT) regulated the two block temperatures separately. Heating rates (0.1–15 °C min^{-1}) and the set-point temperature were continually monitored by a computer with Visual Basic software via a solid-state relay. Two gas channels through the top and bottom aluminium blocks were designed to preheat the gas to avoid influencing the test chamber temperature (Wang, Tang, et al., 2002). Because the heating blocks had low thermal capacitance and high conductivity the resulting temperature profiles were smooth over the heating and holding periods with deviation from the set point temperature (≤60 °C) less than 0.3 °C (Gazit et al., 2004; Yan et al., 2014). Detailed descriptions of the HBS can be found in Ikediala et al. (2000), Wang, Tang, et al. (2002), Johnson et al. (2003), and Yin, Wang, Tang, and Hansen (2006).

2.2. Gas tightness of the CA–HBS system

Because of possible gas leaks from the many connections within the gas delivery lines as well as the seal between the top and bottom heating block, unstable gas concentrations in the insect chamber of lab-scale CA–HBS could result. Therefore, it was necessary to determine gas tightness of the CA–HBS to ensure that the target gas concentrations were maintained (Navarro, 2006). The CO_2 and O_2 flow meters were

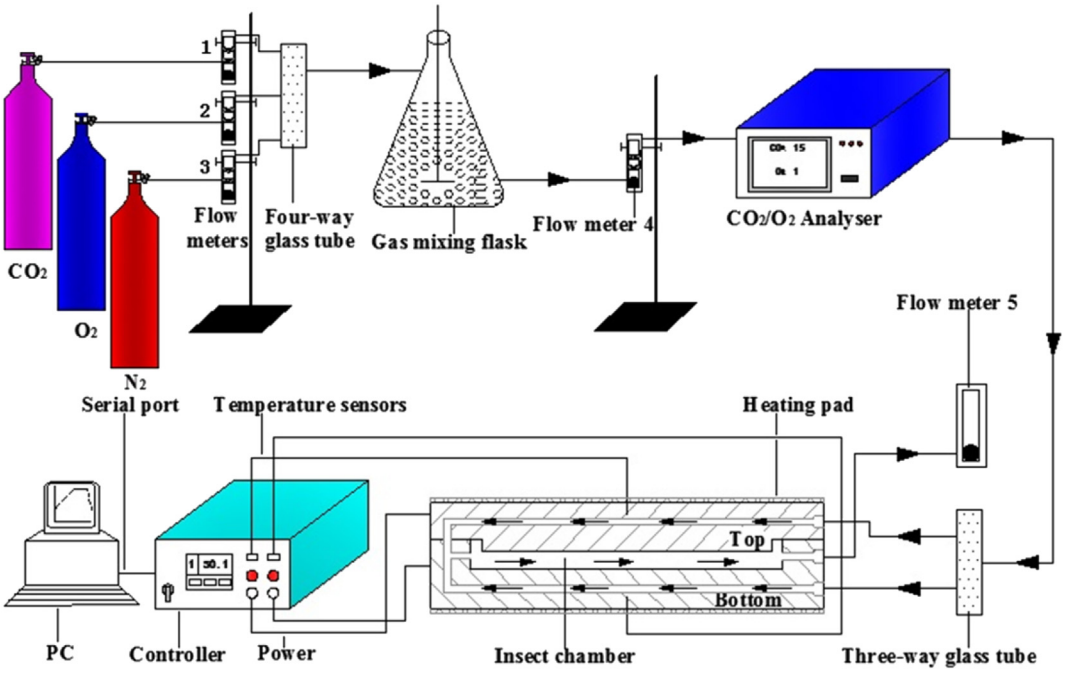

Fig. 1 – Diagram of controlled atmosphere/heating block systems, including gas cylinders, flow meters, gas mixing flask, O_2/CO_2 analyser, and heating blocks with computer controlling and monitoring.

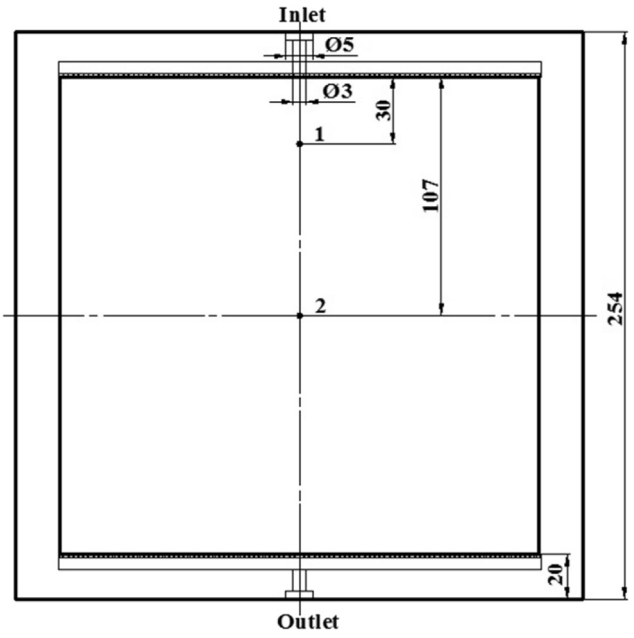

Fig. 2 – Diagram of temperature measurement points located on the bottom block surface (all dimensions are in mm) relative to gas inlet and outlet positions.

set at 200 ml min^{-1} and 20 ml min^{-1}, respectively. The N_2 flow meter was adjusted to 180, 280 and 380 ml min^{-1} to obtain flow rates for the resulting gas mixture of 400, 500, and 600 ml min^{-1}, respectively. Air changes per hour could be calculated by the following equation:

$$N = \frac{60Q}{Vol} \quad (1)$$

where N is number of air changes per hour, Q is volumetric flow rate of air (m^3 min^{-1}), and Vol is volume of the chamber calculated by $L \times W \times H$ (m^3). Relative leakage was obtained based on the readings of the fourth and fifth flow meters. The values of all flow meters were recorded once every 5 min throughout each test, and each test was replicated three times.

2.3. Stability of controlled atmosphere levels

2.3.1. Gas sampling entering the HBS

Gas concentration within the treatment chamber could be influenced by the gas tightness of the system and the accuracy of the O_2/CO_2 gas analyser. Because most CA treatments are low O_2 and high CO_2 concentrations, the stability of gas concentrations going into the insect treatment chamber was analysed at three set point levels: 1% O_2:15% CO_2, 2% O_2:17% CO_2 and 2% O_2:20% CO_2. The gas levels were obtained by adjusting the three flow meters based on the reading from the gas analyser. The electric mixer was run to ensure the gas evenly mixed during the tests. The CA concentration–time profile was obtained by sampling every 5 min over 60 min. Each gas level was replicated three times. The average values and standard deviations were calculated based on three replicates for each test.

2.3.2. Gas sampling exiting the HBS

To determine that gas concentrations within the gas chamber remained stable, samples were also taken of the gas after it exited the chamber. Gas concentrations entering the system were analysed until concentrations reached and maintained the set-point level of 1% O_2:15% CO_2. After a stable set point was reached, samples of the gas entering the system were taken every 3 min for 30 min and then samples of the gas exiting the system was taken every 3 min for 30 min. The time to re-establish the target set point level in the insect chamber was $\leq$6 min after the O_2/CO_2 gas analyser was moved to sample the exiting gas. The average values and standard deviations were calculated based on three replicates.

2.4. Temperature of P1 and P2 in the treatment chamber

2.4.1. Measurement methods

As shown in Fig. 2, two positions (P1 and P2) on the bottom block surface were selected to observe temperature variations, because these positions were in the area most affected by airflow through the inlet and outlet. Surface thermocouples (SA1-T, Omega Engineering Ltd., CT, USA) and data acquisition system (CR-1000, Campbell Scientific. Inc, Logan, Utah, USA) were used for temperature measurements. The temperature variations were analysed under different gas channel designs and block heating rates.

2.4.2. Gas channel design

The effect of two gas channel designs on temperature variation of the HBS block surface was evaluated. In one design, a short channel allowed gas to directly enter the treatment chamber (short channel) (Fig. 3a). In the second design, channels passed the gas through the top and bottom blocks before allowing it to enter the treatment chamber (long channel) (Fig. 3b). In the long channel design, the gas was preheated by passing through the heating blocks before entering the treatment chamber. In both designs the gas stream flowed out of the treatment chamber from an exit port opposite the chamber entry point. Flow rates of 1000 and 470 ml min^{-1} were used in the CA–WB system (Neven, 2008)

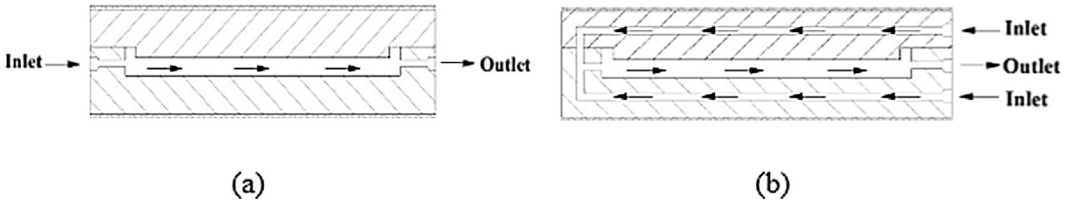

Fig. 3 – Diagram of the gas channel designs with (a) short channel and (b) long channel.

and the CA–HB system (Neven et al., 2012), respectively. Consequently, a combined flow rate of 600 ml min^{-1} (20, 200 and 380 ml min^{-1} of O_2, CO_2, and N_2, respectively) was used to compare temperature variations in the two gas channel designs with variations occurring when no gas (0 ml min^{-1}) was added. A heating rate of 5 °C min^{-1}, starting temperature of 20 °C and set-point temperature of 50 °C was selected for the experiment. All tests were continued for 16 min after the heating block reached the set points.

2.4.3. *Different heating rates*

To determine the effect of heating rate, temperatures at P1 and P2 were recorded under three heating rates (1, 5 and 10 °C min^{-1}) with both gas channel designs and a combined flow rate of 600 ml min^{-1} (20, 200 and 380 ml min^{-1} of O_2, CO_2, and N_2, respectively). A starting temperature of 20 °C and a final temperature of 50 °C were selected with the set-point temperature held for 2 min after it was reached. Each heating rate-gas channel design combination was replicated three times.

2.5. *Applications of CA–HBS to evaluate insect mortality*

After determining the performance of CA–HBS, this system was finally applied 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. A starting temperature of 26 °C and heating rate of 5 °C min^{-1} were used for this comparison test under a gas flow rate through the CA–HBS between 460 and 490 ml min^{-1}. Five actively moving adult *S. zeamais* were exposed to 48 °C for 10, 20, and 30 min. At the end of each treatment, test insects were then removed from the CA–HBS 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. The tests were replicated three times.

Table 1 – Flow rate values (mean ± SD) taken at room temperature and estimated gas leakage within the CA–HBS.

Flow rate (ml min^{-1})			Relative leakage
N_2 (meter 3)	Entering HBS (meter 4)	Exiting HBS (meter 5)	
180	400 ± 0	390.67 ± 1.15	2.33%
280	500 ± 0	490.33 ± 1.53	1.93%
380	600 ± 0	589.08 ± 2.43	1.82%

3. Results and discussion

3.1. *Gas tightness*

Table 1 shows the relative gas tightness of the CA–HBS based on flow meter readings. There was no obvious gas leakage up to the point the gas entered the heating block because the flow rate for meter 4 matched the set-point value. However, the flow rate of the gas leaving the heating block was reduced and the average difference between the flow rates was 9.97 ml min^{-1}, indicating that some leakage had occurred. Relative leakage, probably caused by an imperfect seal between the two block surfaces, was less than 2.4% and decreased with increasing flow rate. The maximum leakage was estimated to be 2.4 air changes per hour. Although this is much higher than the 0.0012 air changes per hour recommended for commercial CA storage by Pflug and Dewey (1959),

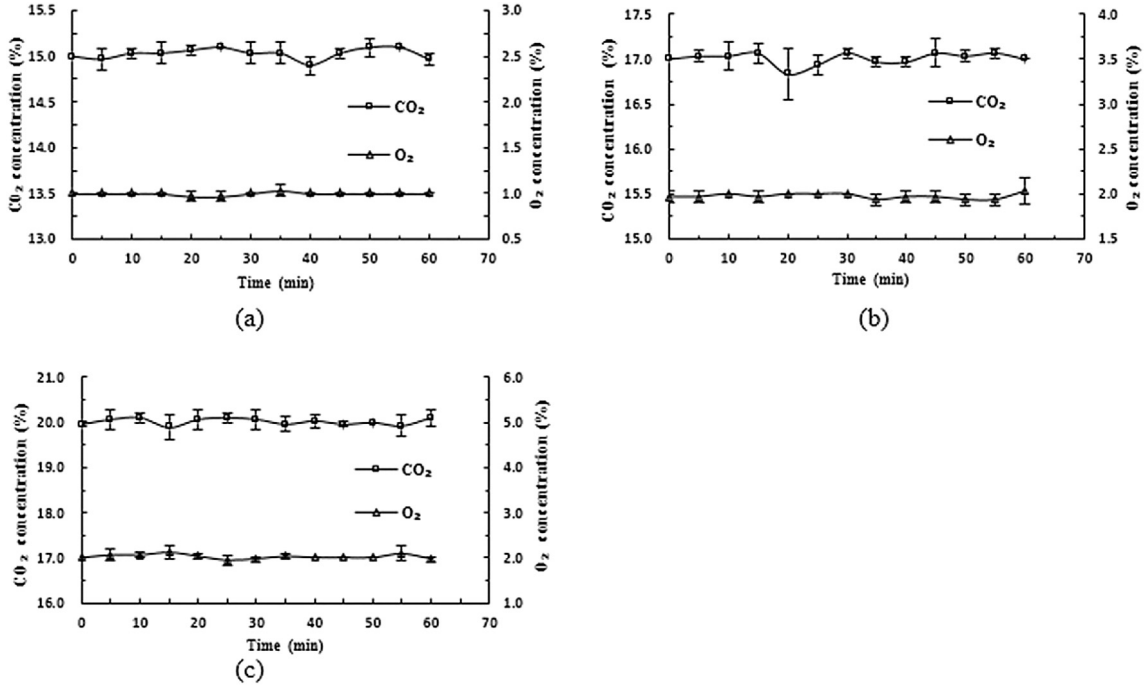

Fig. 4 – Gas concentrations measured by O_2/CO_2 analyser at room temperature and three set-point concentrations: (a) 1% O_2 and 15% CO_2, (b) 2% O_2 and 17% CO_2, and (c) 2% O_2 and 20% CO_2.

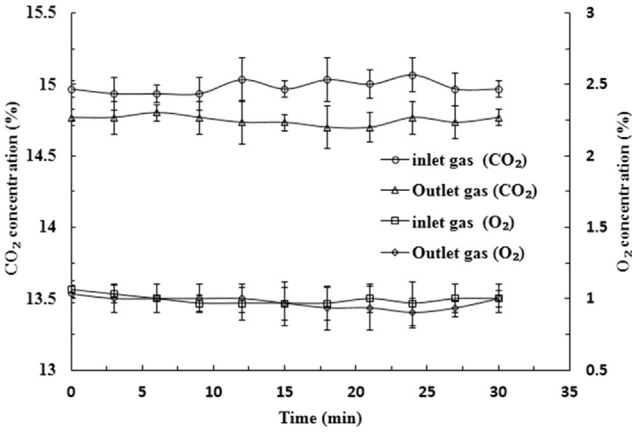

Fig. 5 – Comparison of gas concentrations between coming into and going out the HBS at room temperature and the set-point concentrations of 1% O_2 and 15% CO_2.

it is in good agreement with the result measured in the similar CA–HBS by Neven et al. (2012).

3.2. *Stability of O_2 and CO_2 concentrations*

Figure 4 shows the variation in gas concentration overtime at room temperature. At the three gas set points, O_2 concentrations were more stable than CO_2, with variations within ±0.067% and ±0.167%, respectively. Gas concentrations were more stable in the new CA–HBS when compared with the variations from set point (0.1% for O_2 and 0.5% for CO_2) recorded from a similar system (Neven et al., 2012). Mean concentrations (± standard error, SE) over 60 min were 0.997 ± 0.016%, 1.974 ± 0.031%, and 2.023 ± 0.057% for O_2 and were 15.028 ± 0.059%, 17.005 ± 0.068%, and 20.021 ± 0.069% for CO_2 at the set-points of 1% O_2:15% CO_2, 2% O_2:17% CO_2 and 2% O_2:20% CO_2, respectively. Due to the adjustment being manual, insect mortality tests should begin after reaching stable set point concentrations.

Figure 5 compares concentrations of the gas entering and exiting the chamber over time. The average difference in O_2 concentrations entering and exiting the system was small (0.021%). CO_2 concentrations entering the system were higher than concentrations exiting the system, resulting in an average difference of 0.233%. This is less than the variation of 0.5% found in Neven et al. (2012) and suggests that the gas leakage in this study was negligible.

3.3. *Temperature stability*

3.3.1. *Effect of gas channel design on temperature variation*

Figure 6 shows the temperature at positions P1 and P2 when heated at 5 °C min^{-1} from 20 °C to 50 °C with and without added gas (600 ml min^{-1}) under the two gas channel designs. In the short channel design (Fig. 6a and b), the temperatures of the inlet and centre area with added gas were significantly

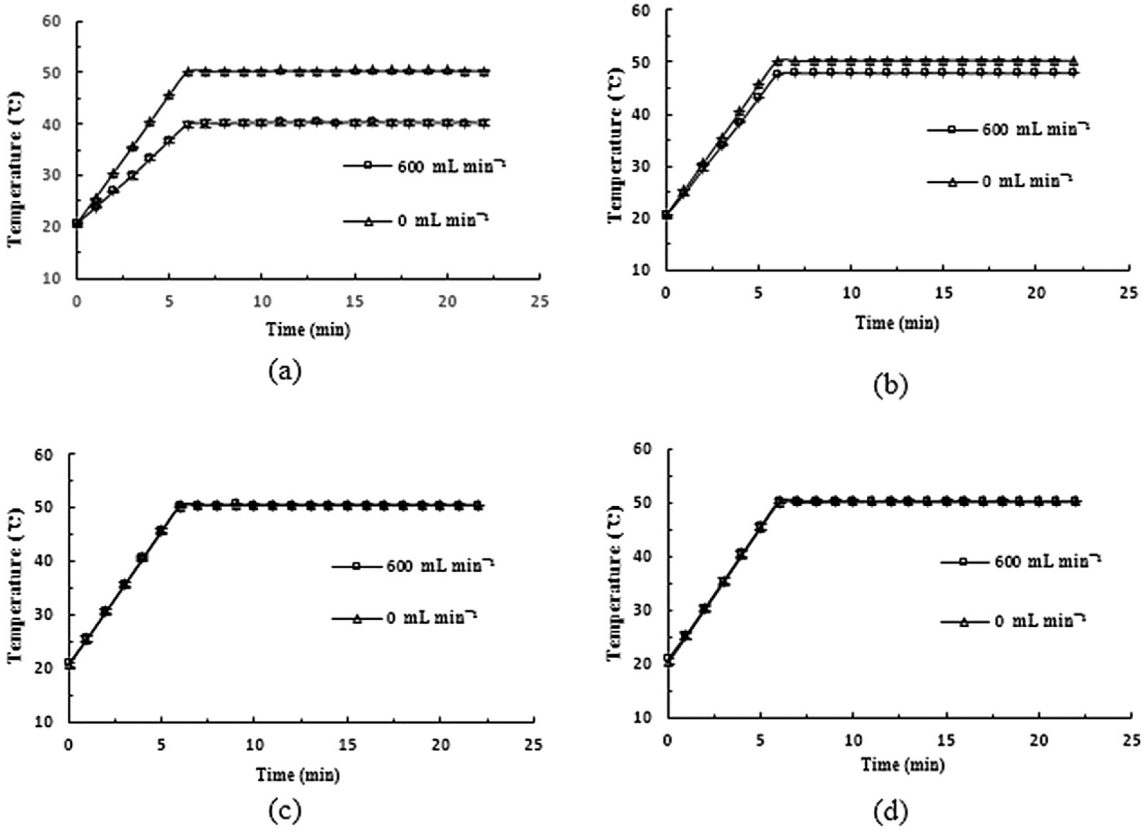

Fig. 6 – Temperature–time profiles near the gas inlet (P1) with short channel (a) and long channel (c) designs, and at the centre of the treatment chamber (P2) with short channel (b) and long channel (d) designs under given gas flow conditions.

lower than with no added gas. When gas was added, the maximum temperature at P1, 30 mm from the gas inlet, reached only 39.63 °C, resulting in a temperature difference of 10.37 °C from the set-point and an actual heating rate of 3.19 °C min^{-1} (Fig. 6a). Because P2 measured temperatures further from the inlet (107 mm), the effect of added gas was reduced, with a temperature difference of 2.54 °C from the set-point and a heating rate of 4.49 °C min^{-1} (Fig. 6b). Therefore, in the short channel design, the addition of un-heated gas had a great effect on the temperature uniformity and heating rate in the treatment chamber, which is not optimal for insect thermotolerance tests.

In the long channel design (Fig. 6c and d), there were no clear differences with or without the addition of gas. When the temperature of the heating block reached the set-point temperature, the temperatures in the inlet (P1) and centre (P2) with and without added gas reached the set-point at the same time. The heating uniformity in the treatment chamber using the long channel design was better than that using the short channel design, indicating that passing the channels through the top and bottom blocks sufficiently preheated the gas, even at a flow rate of 600 ml min^{-1}. Therefore, the long channel design should be used in subsequent insect thermotolerance tests under CA conditions.

3.3.2. *Effect of heating rates on temperature variation*

Figure 7 shows the effects of heating rates (1 °C min^{-1}, 5 °C min^{-1}, and 10 °C min^{-1})in both gas channel designs on temperature variations at P1 and P2 when heated from 20 to 50 °C with an inlet flow rate of 600 ml min^{-1}. For the short channel design results were similar to that found in the previous section (Fig. 6a and b). Temperatures at the inlet (P1, Fig. 7a) and centre (P2, Fig. 7b) did not reach the set-point temperature at any of the heating rates. Maximum temperatures were similar for all heating rates, indicating that the added gas controlled heat transfer in this case. When heating rates of heating blocks were set at 1 °C min^{-1}, 5 °C min^{-1}, and 10 °C min^{-1}, the heating rates near the inlet area (P1) were 0.63 °C min^{-1}, 3.19 °C min^{-1}, and 6.00 °C min^{-1}, respectively, while those near the centre area (P2) were 0.90 °C min^{-1}, 4.46 °C min^{-1}, and 8.68 °C min^{-1}, respectively.

For the long channel design (Fig. 7c and d), there were no clear differences between the programmed heating rates and those calculated from the measured temperatures at either the inlet (P1, Fig. 7c) or the centre of the block (P2, Fig. 7d). This shows that with the long channel design, heating rates had no effect on the temperature in the treatment chamber despite the addition of gas, and confirms previous observations that the long channel design should be used to determine insect thermotolerance under CA conditions.

3.4. *Insect mortality to 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. 8). In all treatments, mortality was higher in heated CA than under heat treatment, with a sharp increase in

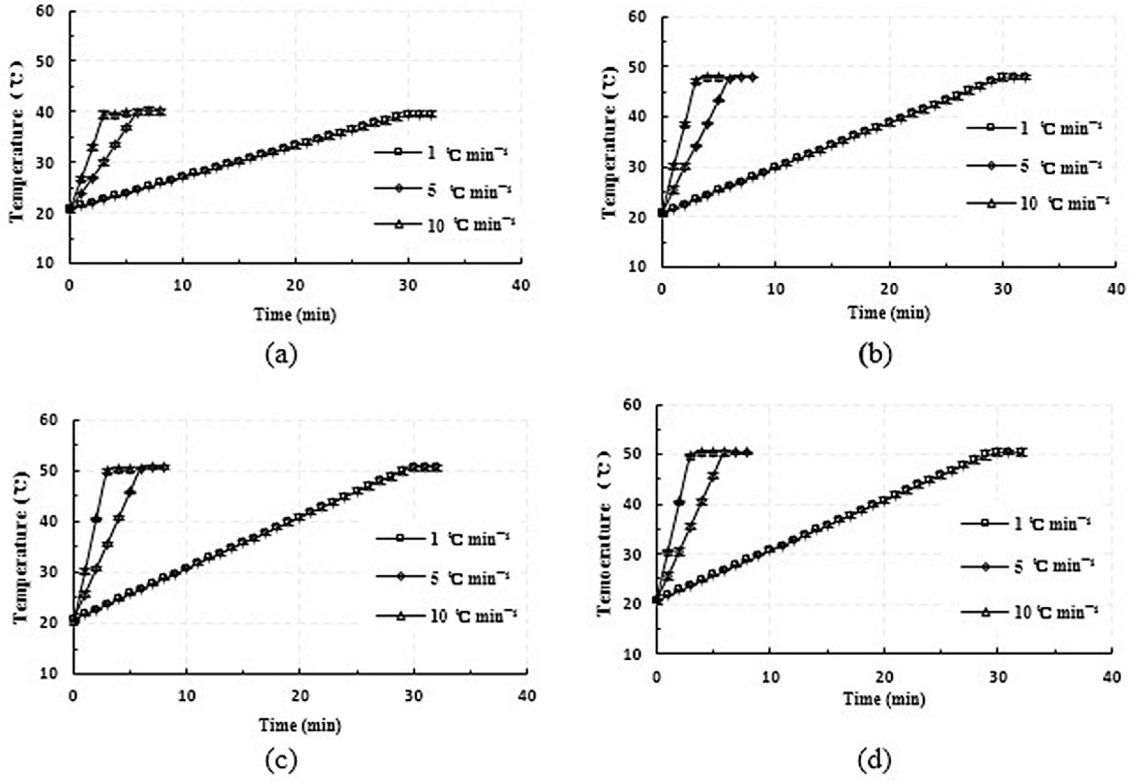

Fig. 7 – Temperature–time profiles near the gas inlet (P1) and at the centre of the chamber (P2) with a gas flow rate of 600 ml min^{-1} and three heating rates.(a) P1 with short channel design; (b) P2 with short channel design; (c) P1 with long channel design; (d) P2 with long channel design.

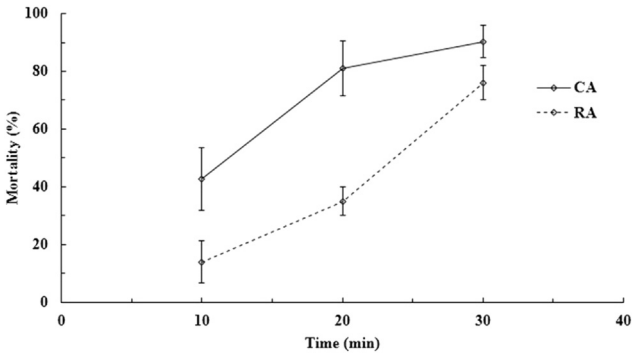

Fig. 8 – Comparison of mean (±SD) mortality (%) of *Sitophilus zeamais* after regular air (RA) (dot line) and controlled atmosphere (CA, 1% O_2:15% CO_2) (solid line) heat treatments at 48 °C with three exposure times at a heating rate of 5 °C min^{-1}.

mortality in the heated CA treatments as exposure increased. 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. Exposure time was significantly reduced in high-temperature treatments of *T. castaneum* when controlled atmospheres were added (Soderstrom, Brandl, & Mackey, 1992). Low oxygen treatment at high temperature was shown to have a more rapid effect on stored product pests when compared to ambient air (Hashem & Reichmuth, 1994). The CA–HBS could be used as an effective device to evaluate insect mortality under combined heat and CA conditions.

4. Conclusions

The recorded performance of the CA–HBS suggests that it would be useful in assessing insect mortality response to high temperature controlled-atmosphere treatments, as well as improving treatment efficiency and reducing treatment costs. The test of gas tightness showed that the insect treatment chamber had a stable and low leakage rate. The new CA–HBS had the ability to control the added gas composition and maintain relative stable concentration at set-point levels. Temperature data from the treatment chamber showed that final temperatures and heating rates could reach targeted values even under a relatively high gas flow rate, as long as gas was preheated in the long gas channel design. The reasonable insect mortality to heated CA conditions showed that the CA–HBS could be used as an effective and reliable device to rapidly assess insect responses under combined heat and CA conditions.

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