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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*.

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