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Low oxygen concentration hinders the occurrence of the cigarette beetle, *Lasioderma serricorne* under nitrogen-controlled atmosphere storage system

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Abstract

Cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae) can infest a wide range of stored products such as food and cereal grains, and it is also particularly common on the infestation of tobacco. Fumigants, such as phosphine and sulfuryl fluoride have been widely used to control this insect pest, but some serious drawbacks, such as insect resistance to the fumigants and damage to the environment due to the excessive application, have limited their applications. Environmentally friendly alternatives to replace these chemical insecticides to control this pest are urgently required. Controlled atmosphere treatment such as using high concentration nitrogen (N_2) is an alternative to control this notorious pest. In this study, we investigated the mortality of different stages of cigarette beetles at different oxygen (O_2) concentrations (≤2 or 2 to 4%) by applying high concentration N_2 (≥99.95%) to a tobacco warehouse. The results revealed that the corrected mortality of eggs, larvae, pupae and adults was 100% under the low O_2 (≤2%) concentration after 27 d exposure. The corrected mortality of larvae exceeded 85% and hatching rate was less than 10% after 27 d when O_2 concentration was 2 to 4%. Thus, the result suggested that N_2 controlled atmosphere treatment was an environmentally friendly method to manage this stored tobacco pest.

Keywords: *Lasioderma serricorne*, Nitrogen treatment, Control atmosphere storage, Stored pest control

Introduction

The cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), is an economically important storage pest worldwide (Edde, 2019). The species is destructive and infests many stored products, such as tobacco, cereal grains and herbs (Cao et al., 2019). In particular, the infestation of cigarette beetle causes huge economic losses to the tobacco industry around the world. Several technologies have been developed to control *L. serricorne*, such as heat or cold treatments, biological control, fumigation, and radiation (Oliveira da Silva et al., 2018).

The common and effective control method is the phosphine fumigation (Oliveira da Silva et al., 2018; Edde, 2019). However, the excessive usage of phosphine has resulted in insect resistance to this fumigant and environmental pollution (Saglam et al., 2015; Li et al., 2018; Yang et al., 2019). Therefore, environmentally friendly alternatives for control of this pest are urgently needed.

Storage insect pests are animals requiring oxygen for their survival. Controlled atmosphere treatment (CA) offers an alternative that is safe and environmentally benign to the treated materials (Navarro, 2012). During the CA treatment, oxygen concentrations within a storage enclosure can be altered to the lethal O_2 concentration to the insect pests by adding N_2 or CO_2 (Navarro, 2012; Edde, 2019). The efficiency of CA depends on temperature, relative humidity (RH) and life stage of insects (Edde, 2019). Nitrogen controlled storage system has had a broad application after undergoing various optimizing processes and cost reductions in China (Zhang et al., 2015; Xiao et al., 2019). In recent years, several grain-storage companies have applied N_2 to control pest insects (Lin, 2019). Several studies have shown that high N_2 concentration in storage systems can effectively inhibit pest population increase (Lu et al., 2013; Liu, 2016; Xiao et al., 2019). In a study on the mortality of *L. serricorne* under the nitrogen treatment (O_2 concentration ≤2%), no live cigarette beetles were found on different linings of tobacco leaves after 45 d exposure (Xiao et al., 2019). Another report also showed that the mortality of the cigarette beetle adults and larvae could reach 100% under low O_2 (1% or 2%) and high temperatures (Liu, 2016). The effect of 98% nitrogen on the survival of other stored pests has also been studied (Lu et al., 2013).

In this study, we investigated the survival of different stages of cigarette beetle under low O_2 concentrations by applying high concentration N_2 (≥ 99.95%) into a tobacco warehouse. Our results could provide valuable information for developing an effective technique to control *L. serricorne*.

Materials and methods

Insects

The *L. serricorne* was provided by Zhengzhou Tobacco Research Institute of China National Tobacco Corporation. The culture was reared on whole wheat flour mixed with yeast (7:3, *w*/*w*) and maintained at 29 ± 1°C and 75% ± 5% RH under dark conditions.

Tobacco warehouse

The re-roasted and processed tobacco leaves from Yunnan province were stored in a warehouse located at the Nanjing cigarette factory, China and kept at 18 to 26°C and 55 to 65% RH. The tobacco stacks (14 m × 4.3 m × 3 m) were sealed with 0.12 mm nylon film on six sides of the stacks for air tightness.

Nitrogen treatment

The airtightness of the sealed stacks was tested before nitrogen application, and all the stacks reached the Chinese airtightness standard (half-life time of the pressure ≥ 5 min from 300 to 150 Pa). Highly purified nitrogen (≥99.95%) was produced from the air through zeolites by pressure swing adsorption in the nitrogen generation equipment (FD-PSA-100, Wuhan Dongchang Storage Technology Co. Ltd., China). The desired oxygen concentration (≤2% and 2 to 4%) was obtained by adding the produced N_2 (≥99.95%) to the stacks in the tobacco warehouse (26°C and 65% RH) (Fig. 1).

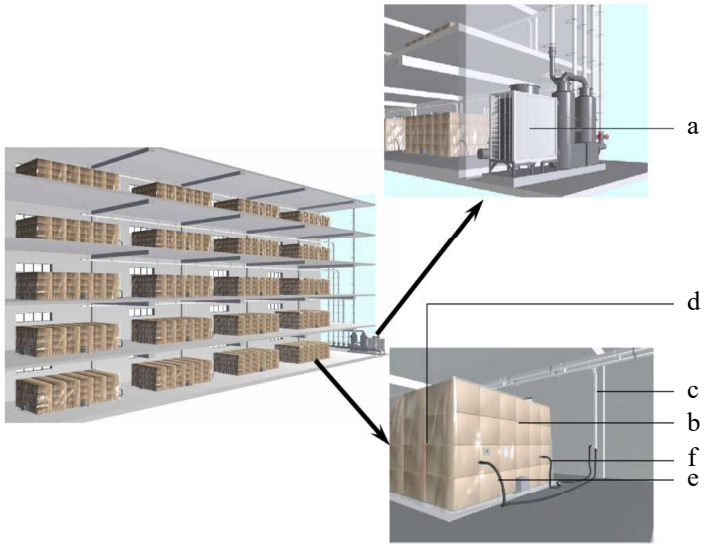

Fig. 1. Schematic diagram of nitrogen treatment of tobacco stacks: (a) Nitrogen generation device, (b) Tobacco stack, (c) Nitrogen filled/exhaust ducts, (d) Temperature/oxygen concentration probe, (e) Nitrogen filled ducts, and (f) Nitrogen exhaust ducts.

Treatment of insects

Different stages (eggs, larvae, pupae and adults) of *L. serricorne* were exposed to low oxygen (O_2) concentrations (≤2%, 2%-4%) under a controlled high N_2 (≥99.95%) atmosphere in the tobacco warehouse. The air (21% O_2) served as the control. The tested insects in plastic vials (8 cm × 12 cm) were located at 10-15 cm below the surface of tobacco stacks. The mortality of the treated insects (eggs, larvae, pupae and adults) was observed after 18, 21, 24, 27, 30 and 33 d exposure. The larvae or adults were considered dead if no movement was observed. The plastic vials which held the *L. serricorne* pupae were maintained in an incubator (29 ± 1°C and 75% ± 5% RH) after the low O_2 treatments, and the number of pupae which developed into adults was recorded daily for the following 7 d. The pupae that could not develop into adults were considered dead. The treated eggs were also maintained at the insect rearing condition after the treatment. The number of eggs which hatched into larvae was recorded daily for the following 7 d. The eggs that could not develop into larvae were considered dead. Three replicates were conducted for each treatment.

Results

Mortality of L. serricorne eggs, larvae, pupae and adults under normal O_2 concentration (21%)

We observed the survival of *L. serricorne* eggs, larvae, pupae and adults at normal O_2 concentration during the experiment (Fig. 2). The results showed that the mortality rate of eggs was the highest (34.4%) among the four stages. The larval mortality was 13.1% under the normal condition. For the pupae and adults, the mortalities were 3.1% and 6.3%, respectively.

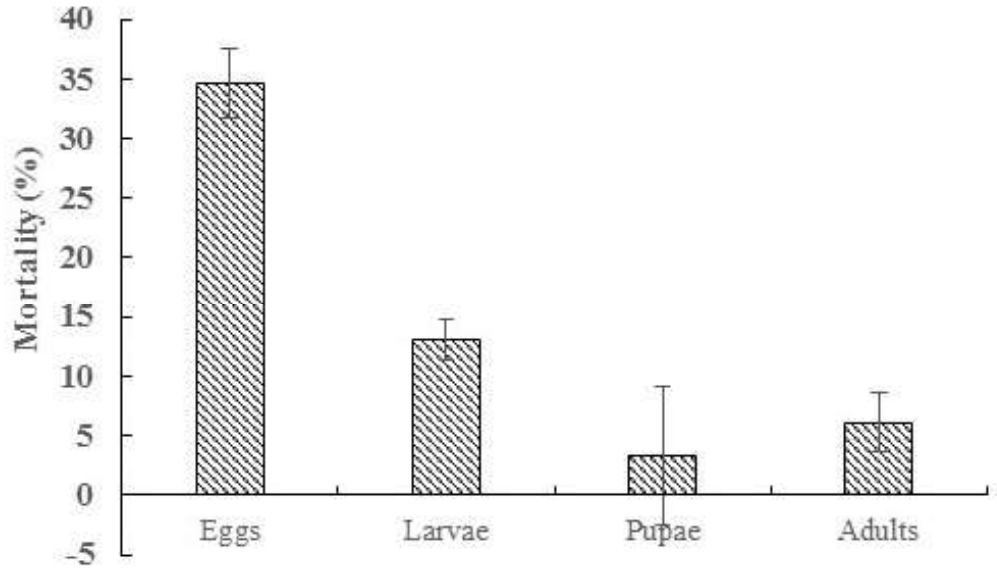

Fig. 2 Mortality of different stages (eggs, larvae, pupae and adults) *L. serricorne* at control condition (air with 21% O_2).

Effect of ≤2% O_2 on the survival of eggs, larvae, pupae and adults

When exposed to ≤2% O_2 for 18, 21 and 24 d, the corrected mortalities of eggs were 84.2, 92.7 and 99.0%, respectively, while larvae had mortalities of 89.6%, 95.3% and 98.6%, respectively (Fig. 3). The corrected mortality of eggs and larvae reached 100% under the low O_2 condition after 27 d. However, no live pupae and adults were found after 18 d under ≤2% O_2 (Fig. 3).

$$\text{Corrected mortality (\%)} = \frac{\text{Treatment mortality} - \text{Control mortality}}{1 - \text{Control mortality}} \times 100\%$$

Effect of 2 to 4% O_2 on the survival of eggs, larvae, pupae and adults

The highest corrected mortality of eggs and larvae were 93.5% and 87.6%, respectively, when exposed to 2 to 4% O_2 within 33 d (Fig. 4). For the pupae, the corrected mortalities were 97.1% and 99.5% when exposed to the low oxygen condition for 18 and 21 d, respectively. Live pupae were not observed after 24 d under any treatment (Fig. 4). The corrected mortality of adults was 99.4% under 2 to 4% O_2 treatment for 18 d, and reached 100% after 21 d (Fig. 4).

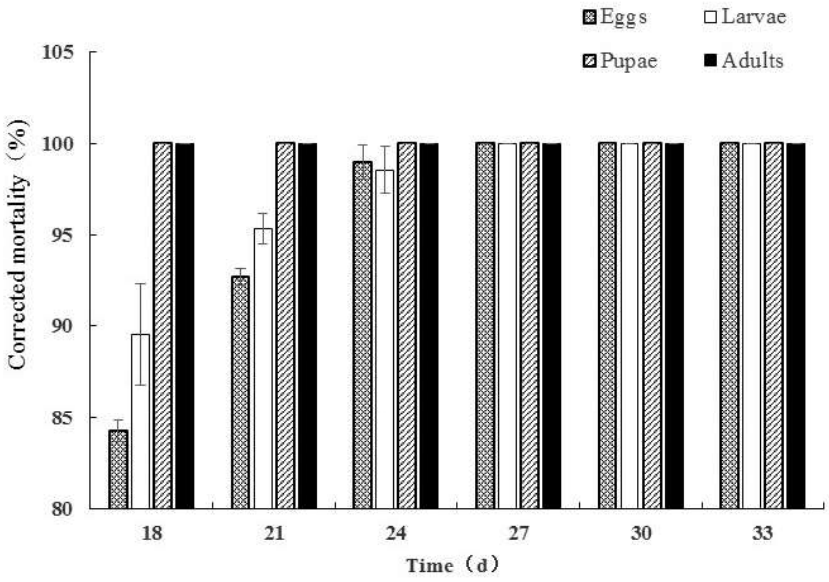

Fig. 3. Corrected mortality of different stages (eggs, larvae, pupae and adults) of *L. serricorne* under ≤2% O_2.

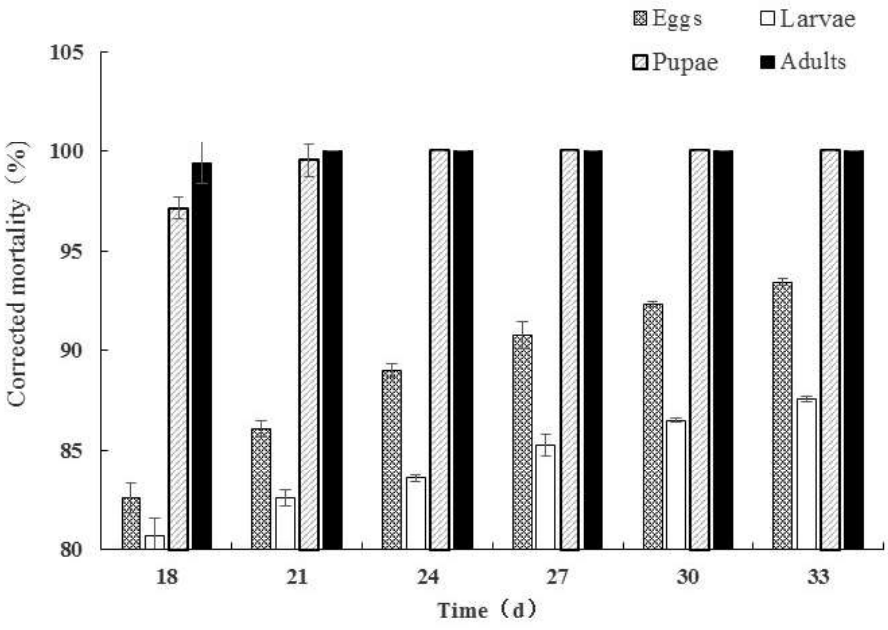

Fig. 4. Corrected mortality of different stages (eggs, larvae, pupae and adults) of *L. serricorne* under 2 to 4% O_2.

Discussion

In this study, eggs, larvae, pupae and adults of *L. serricorne* were exposed to low oxygen concentrations. The results indicated that low oxygen treatments (≤2%, 2%-4%) were effective in the control of different life stages of *L. serricorne*. The mortality of eggs, larvae, pupae, and adults was 100% under the low O_2 (≤2%) concentration after 27 d. No live pupae and adults were observed under 2 to 4% oxygen concentrations after 24 d; however, different stages of tobacco beetle had different tolerance to the low oxygen. When oxygen concentration was less than 2%, the mortality of eggs was 84.2% after 18 d, while pupae and adults had 100% mortality. Our data clearly showed that eggs were the most tolerant stage under the low oxygen concentration (≤2%). The tolerance of the four stages of *L. serricorne* to the low oxygen concentration (≤2%) in increasing order was adults, pupae, larvae, and eggs. When oxygen concentration was 2 to 4%, the larval stage had the highest tolerance because many larvae (12.4%) were still alive after 33 d. The tolerance of the four stages of *L. serricorne* to 2 to 4% oxygen concentration in increasing order was adults, pupae, eggs, and larvae. In conclusion, the low oxygen concentrations (≤2% or 2 to 4%) were effective to control *L. serricorne*.

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