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Exposure time prediction of N_2 treatment based on behavior and mortality of *Sitophilus oryzae* (L.) adults monitored by camera at different temperatures

Dianxuan Wang[1]*, Yilin Huang[1], Chunqi Bai[1], Jianhua Lu[1], Fuji Jian[2]

[1]Henan University of Technology, Collaborative Innovation Center of Henan Grain Crops, Henan Collaborative Innovation Center of Grain Storage and Security, Zhengzhou, 450001, China.
[2]Biosystems Engineering, University of Manitoba, Winnipeg, MB R3T 2N2, Canada.
*Corresponding author email: wangdx@haut.edu.cn

Abstract

The effect of controlled atmosphere (CA) on insects is usually evaluated by sampling and further incubating. This sampling and incubating method cannot monitor the control effect before the control is completed. Behaviour of insects in cages can be monitored by using video camera equipment. The knockdown time of *Sitophilus oryzae* (Linnaeus) adults monitored by a video camera located in an airtight chamber filled with 98% nitrogen was determined at 18, 23, and 28℃. The relationship between the exposure time, the knockdown percentage, and mortality was analyzed. It was 18, 16 and 10 d from the time of 50% knockdown to the time of 100% mortality at 18, 23, and 28℃, respectively. The equations describing the knockdown percentage (x) and the time difference from the knockdown to 100% mortality of adults (y) were $Y = -11.872x + 23.369$ ($R^2 = 0.9736$), $Y = -7.8218x + 18.678$（$R^2 = 0.856$), and $Y = -5.1382x + 12.402$ ($R^2 = 0.9659$) at 18, 23, and 28℃, respectively. The most tolerant life stage of *S. oryzae* was that of the pupa. The population extinction time could be predicted by using the developed regression equations associated with the most tolerant life stage under the tested conditions.

Keywords: High concentration nitrogen, *Sitophilus oryzae*, Knockdown percentage, Exposure time prediction, Controlled atmosphere storage

Introduction

The application of high concentration nitrogen to control stored grain insect pests has been used in many countries (Annis, 1986; Navarro, 2006; Carvalho, 2012; Cai, 2019; Moncini et al., 2020; Sule and Emekci, 2020; Lorenzo et al., 2020). Commercially available equipment called the ‘‘pressure-swing adsorption’’ system (PSA) uses the process of O_2 adsorption from compressed air passed through a molecular sieve bed. The system provides about 120 m^3/h of 98% N_2 (Navarro, 2012) and has been used for more than 15 million tonnes of stored grain in China (Yang et al.,

2016). The effect of this controlled atmosphere (CA) treatment is typically verified by inspecting the insect survival rate after this treatment process has been completed. Another method used to verify the killing effect of CA treatment in the field studies is to observe insect behaviour in cages or enclosures (Meenatchi et al., 2016). If some individuals survive in the enclosures, the CA treatment is considered a failure. The knockdown and death of insects in the CA enclosures are influenced by many factors including temperature, relative humidity, insect species, and life stages (Soderstrom et al., 1986; Donahaye,1996; Ofuya and Reichmuth, 2002; Gunasekaran and Rajendran, 2006; Hashem, 2012, Huang et al., 2020a). Temperature is the key factor, and low temperatures can extend the insect survival time (Huang et al., 2020a, b). Therefore, monitoring the insect behaviour continually in the enclosures is required due to the temperature fluctuations in storage structures. A convenient method is to use a high-resolution video camera to remotely monitor these small individual insects in the enclosures (Gavankar, 2013). In this study, the behaviour of *Sitophilus oryzae* at 18, 23, and 28°C and in 98% N_2 was monitored by using such a camera. The knockdown percentage of the adults was calculated. The relationships between the knockdown percentage and population extinction time were analyzed. This relationship was used to predict the exposure time needed in the field treatment.

Materials and methods

Insects and treatments

The *S. oryzae* was originally collected from a grain depot located in Nanning, Guangxi Zhuangzu Municipality, China and reared for five years at 28±1°C and 75±5% r.h. on wheat. More than 1,000 adults of mixed sex were transferred to a 1 L jar containing 800 g of wheat kernels for egg laying purposes. The adults were sieved out after 24 h. The wheat and eggs were kept at 28±1°C and 75±5% r.h. After another 24 h, the wheat kernels with the eggs were further divided into 5 g samples. This procedure was repeated for preparation of adults, pupae, and larvae after 42, 22, and 12 d, respectively. These time periods were calculated from the known *S. oryzae* development time at the rearing condition (Huang et al., 2020b).

Gas chamber and high N_2 management

The concentration of nitrogen with air mixture was maintained in a rectangular gas chamber with dimensions of 60×35×40 cm^3 (Fig. 1). The gas chamber was made of 2 mm armor plate, except for the top side, which was made of 2 mm transparent plexiglass. Saturated sodium chloride solution kept in a 250 mL beaker was used to maintain the 75±5% r.h. in the gas chamber. To keep the airtightness of the gas chamber, airproof sealing was used at the bolt and connection locations. The halftime of pressure drop of the chamber at the initial pressure of 500 Pa was 180 s. A cylinder containing compressed liquid of 99.999% N_2 was connected to the chamber by a rubber tube of 6 mm inner diameter (Fig. 1). The outlet from the gas chamber was connected to a recirculating pump (DC24V, Hailin Technology Co., Ltd., Chengdu, China) and an N_2 detector (MOT500—LM(N_2), Kenuoer Co., Shenzhen, P. R. China) using a rubber tube (Fig. 1). At the beginning of the operation, the valve was opened until the N_2 concentration inside the chamber reached 98%. The N_2 concentration in the chamber was then measured by the N_2 detector after the valve was closed. The N_2 detector can measure nitrogen concentrations in the range of 0 to 100%

with a 0.01% resolution. After the N_2 concentration inside the gas chamber was higher than 98%, the valve was closed. When the concentration of N_2 in chamber reached above 98%, the concentration of O_2 was 1.88 to 1.98% as measured by an O_2 detector (MOT500-O_2, Kenuoer Co., Shenzhen, China) capable of measuring O_2 concentrations from 0 to 100% with a 0.01% resolution (Fig. 1).

For video recording, the prepared 50 adults of mixed sex and the 2 g of wheat kernels were introduced into a 60 mm diameter petri dish. The petri dish was covered with nylon mesh (80 mesh) that was hot melted onto the petri dish rim. The petri dishes were installed at the top of the gas chamber and supported by a steel frame (Fig. 1). The nylon mesh was at the bottom so that the adult behaviour could be captured by the camera (HD1800, Sanhao Instrument Equipment Co., Ltd, Shenzhen, China) through the transparent plexiglass and transparent bottom of the petri dish.

Before the N_2 was introduced into the chamber, the petri dishes and insect cages holding 5 g wheat kernels with the adults (and hidden immature stages of insects) were located inside the chamber (Fig. 1). The cages were used to hold insects and grain, so that insects with different exposure times could be moved out of the gas chamber at different times. Each cage was 10 mm in diameter and 70 mm in length. There was one hole on one vertical side of the gas chamber that was sealed with a rubber glove. There was an N_2 gas cylinder on another vertical side that was sealed by two screw caps (80 mm in diameter and 200 mm in length). This hole, the airtight glove, and the cylinder were all used in assisting to remove the insect cages out of the gas chamber during the CA treatment without losing the airtightness of the chamber (Fig. 1). When a cage was removed out of the chamber, the cage was held by using the rubber glove and placed inside the cage removal cylinder. The cylinder was then sealed by turning the internal screw cap A. The cage was moved out of the cylinder once the outer screw cap B was removed. Insect cages were taken out of the CA chamber every 2 d during the 3 to 30 d period of the experiment.

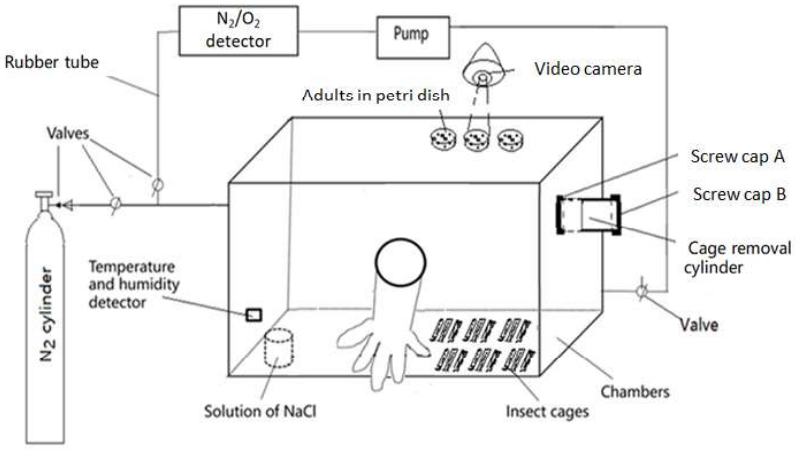

Fig. 1. Diagram of gas chamber and material arrangement

There were three replications for each observation. The control was conducted under the same conditions, except the air was used instead (Fig. 1). The gas chamber was kept inside the lab which was controlled by an air conditioning system at 18, 23, and 28℃ with ±1℃, respectively.

Insect behaviour monitoring and mortality evaluation

The recorded video was replayed on a computer and the knockdown time of each individual was counted. When an adult had no movement of body, antennae or legs, the adult was counted as a knockdown adult. The percentage of knockdown at different treatment times was calculated as: knockdown percentage = 100 × number of knockdown insects / total treated insects. The adult mortality in each cage was counted after 14 d. The mortality of the hidden immature stages of insects was evaluated by counting emerged adults over three successive days. After that, the survival of pupae, larvae and adults in wheat kernels was checked by cutting the kernels. All the tested insects and wheat kernels were kept at 30±1℃ and 75±5% r.h. after they were moved out of the chamber.

Statistical analysis

The time difference between 100% knockdown and 100% mortality was calculated for each stage and at each temperature. The percentages of knockdown and mortality at different times and different temperatures were compared by conducting Tukey's test. Linear regression between the percentage and time at each temperature was conducted. All data analysis was conducted by using IBM SPSS Statistics 20.

Results

Population extinction time of S. oryzae

At any treatment temperature, exposure time of 100% mortality from long to short was pupa > egg > larva > adult (Table 1.). The time of 100% mortality of pupa can be used as the population extinction exposure time. Compared with adults and at 23℃, the time of 100% mortality of pupa, egg, and larva was delayed for 8, 6, and 4 d, respectively. The time of 100% mortality of any stage of the *S. oryzae* increased with the decreasing temperature.

Table 1. Exposure time (d) of 100% mortality of *S. oryzae* in 98% N_2 at different temperatures

Temperature (℃)	Adult	Larva	Egg	Pupa
18	16.67±1.15a	19.00±1.73a	21.00±0.00a	25.00±1.63a
23	11.00±0.00b	15.33±0.81b	17.00±1.00b	19.00±1.00b
28	7.33±0.57c	11.00±1.00c	11.33±1.52c	12.67±0.57c

The different letters (a, b, c) in the same column are significantly different at $\alpha = 0.05$ level using Tukey's test.

Knockdown time and 100% mortality time

The knockdown time (TK) increased from 2.9 to 14.7 d at 18℃, from 2.1 to 9.9 d at 23℃, and from 1.5 to 6.3 d at 28℃, respectively, as the knockdown percentage increased from 10 to 100%. Therefore, knockdown percentage increased with the increase of the treatment time. The 100% knockdown time at 18℃ was more than double of that at 28℃ (Table 2.).

Table 2. Knockdown time (d) of *S. oryzae* adults in 98% N_2 at different temperatures

Knockdown percentage (%)	18℃	23℃	28℃
10	2.89±0.59aA	2.13±0.12bA	1.45±0.18cA
20	3.96±1.25aB	2.47±0.08bB	1.82±0.50cB
30	5.44±0.60aC	2.78±0.10bC	2.10±0.10cC
40	6.98±0.42aD	3.03±0.61bD	2.51±0.11cD
50	7.49±0.83aE	3.49±0.11bE	2.88±0.25cE
60	8.40±0.46aF	4.03±0.21bF	3.45±0.34cF
70	9.07±1.08aG	4.46±0.21bG	3.99±0.08cG
80	10.71±0.36aH	6.08±1.15bH	4.40±0.11cH
90	11.93±0.23aI	7.87±0.65bI	5.35±0.57cI
100	14.73±1.22aJ	9.90±0.61bJ	6.29±1.65cJ

The different letters (a, b, c) in the same row are significantly different. The different letters (A, B, C) in the same column are significantly different. All values are averages ± SE at α = 0.05 level using Tukey's test.

The time difference between knockdown time and 100% mortality decreased with the increase of temperature (Fig. 2). Even after the adults were in complete knockdown, it still required at least 10.3, 9.1, and 6.7 d to reach 100% mortality of the remaining population at 18, 23 and 28℃, respectively. If a knockdown percentage (x) was given, the time difference between TK and 100% mortality (Y) could be calculated as:

$$Y = -11.872x + 23.369,\ R^2 = 0.97,\ 18℃$$
$$Y = -7.8218x + 18.678,\ R^2 = 0.86,\ 23℃$$
$$Y = -5.1382x + 12.402,\ R^2 = 0.97,\ 28℃$$

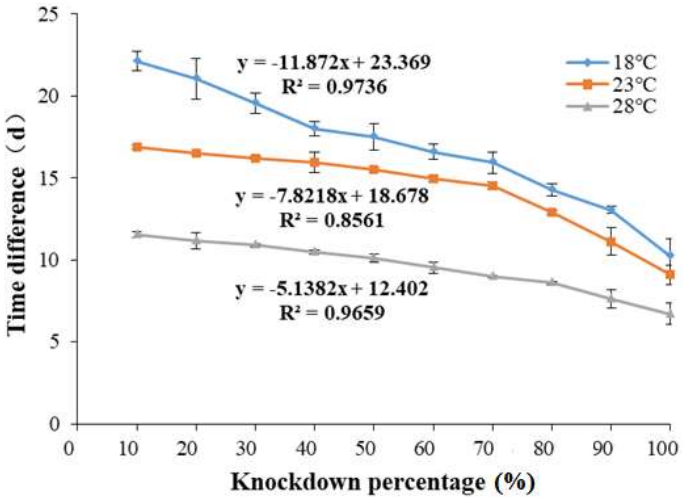

Fig. 2. Time difference between knockdown time and 100% mortality of *S. oryzae* pupae in 98% N_2 at different temperatures.

Conclusions

Temperature had significant influence on different stages of *S. oryzae* when they were treated in 98% N_2 mixed with air, not only for their knockdown time, but also for exposure time of 100% mortality or population extinction exposure time.

At any treatment temperature, exposure time of 100% mortality from long to short was pupa > egg > larva > adult. Even after the adults were in complete knockdown, it still required at least 10.3, 9.1, and 6.7 d to reach 100% mortality of the remaining population at 18, 23 and 28°C, respectively.

It is difficult to check the survival of immature life stages because they hide inside grain kernels. Knowing the response of adults during CA treatment by using a video camera and predicting the population extinction exposure times are important for the control of hidden insects. The equation between knockdown percentage (x) of adults and the time difference between the knockdown and 100% mortality (Y) were:

$Y = -11.872x + 23.369$ ($R^2 = 0.9736$) at 18°C
$Y = -7.8218x + 18.678$ ($R^2 = 0.8561$) at 23°C
$Y = -5.1382x + 12.402$ ($R^2 = 0.9659$) at 28°C

To predict the exposure time required for killing all of the stages, especially for the most tolerant stage of the pupa, using video recordings to monitor the adults' behaviour will be a useful tool to successfully conduct the control atmosphere storage.

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