Evaluation of the respiration rates of *Sitophilus zeamais*, *Rhyzopertha dominica* and *Tribolium castaneum* at three constant temperatures with and without a food source

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Abstract

While carbon dioxide (CO_2) concentration monitoring has been used for spoilage detection and quality management in stored grain, much of the decision making process still relies on the operator's interpretation of CO_2 concentration data. As an initial attempt to use CO_2 concentration readings for quantification of insect population in stored grain, this study aimed at establishing baseline respiration rates of three species of primary stored product insects, namely maize weevil (MW) (*Sitophilus zeamais*), red flour beetles (RFB) (*Tribolium castaneum*) and lesser grain borers (LGB) (*Rhyzopertha dominica*). Changes in CO_2 concentrations due to insect respiration were monitored in a closed system using non-despersive infrared CO_2 concentration loggers. For each experimental replicate, a known number of adult insects were placed along with a CO_2 data logger in a glass desiccator for 15 h, and the respiration rate was calculated from the slope of the CO_2 concentration curve. Three different numbers of insects (i.e., 50, 100 and 200 insects) were tested at three constant surrounding temperatures (i.e., 25, 30 and 36ºC). In addition, respiration rates were measured both when a food source (i.e., 125 g of brown jasmine rice) was and was not provided for the insects. The split-plot experimental design was implemented for each insect species. For all replicates, CO_2 concentrations increased linearly. Regardless of surrounding temperatures, all three insect species respired at higher rates when a food source was provided. When averaging across the insect numbers, without the presence of food the respiration rates of MW, LGB and RFB were 2.58–5.47, 1.04–2.23 and 1.02–2.73 μl_{CO2}/insect h, respectively. Similarly, when food was present, the respiration rates increased to 10.15–13.76, 1.77–4.19 and 4.69–11.33 μl_{CO2}/insect h, respectively. Changes in temperature affected respiration rates significantly. In general, the different numbers of insects used in the experiments did not yield significantly different respiration rates. From the perspective of grain quality management, this study provided important baseline data and experimental protocol for developing decision support tools based on quantitative insect population.

Keywords: respiration rate, carbon dioxide, grain quality, stored product insects

1. Introduction

Cereal grains could be stored for periods of a few weeks to a few years before they are processed. Respiration rates of organisms can be represented as oxygen (O_2) consumption and/or carbon dioxide (CO_2) production rates (Fonseca et al., 2002). In grain storage structures, CO_2 gas is generated by biological activities of the grain itself as well as infesting insects and micro-organisms. In recent years, CO_2 measurement technology for grain quality

monitoring has been developed and improved. However, interpretation of the relationship between CO_2 gas concentrations and the infestation level of insects and/or fungi still relies on the experience of human operators. In addition, few studies have been conducted to quantitatively specify the extent of infestations based on CO_2 concentration readings. Emekci et al. (2002, 2004) measured respiration rates of red flour beetles (RFB) (*Tribolium castaneum*) and lesser grain borers (LGB) (*Rhyzopertha dominica*) adults and showed that when surrounding temperature were 25, 30 and 35°C, the respiration rates of RFB and LGB were 1.68 and 2.19, 2.37 and 3.05, and 3.31 and 5.04 μl_{CO2}/mg h, respectively. Neven (1998) showed that larvae of codling moth (*Cydiapomonella* (L.)) respired at higher rates when surrounding temperature increased. Guedes et al. (2006) found that the respiration rate of maize weevil (MW) (*Sitophilus zeamais*) adults were in a range of 3.24–5.60 μl_{CO2}/insect h. Lu et al. (2009) studied the effect of ozone (0.1, 0.2 and 0.4 µg/ml) on the respiration rates of adults of rice weevils (RW) (*Sitophilus oryzae* (L.)), RFB and LGB. As ozone concentrations increased, the insects respired at slower rates. Furthermore, these researchers found that the respiration rates of RW, RFB and LGB in atmosphere air were 2.00, 1.93 and 2.04 μl_{CO2}/mg h, respectively. When surrounding temperature was below 30°C, respiration rates of *Acarus siro* (L.), *Dermatophagoides farinae* (H.), *Lepidoglyphus destructor* (S.) and *Tyrophagus putrescentiae* (S.) linearly increased as the temperature increased (Hubert et al., 2010). However, an inverse relationship occurred when surrounding temperature was higher than 30°C (Hubert et al., 2010). While Emekci et al. (2002, 2004) and Neven (1998) measured insect respiration rates with presence of a food source, in the experiments of Guedes et al. (2006), Hubert et al. (2010) and Lu et al. (2009) no food source was provided for the insects. This difference in the presence of food could result in variations in the measurements. As an initial attempt to use CO_2 concentration readings for quantification of insect population in stored grain, the objective of the present study was 1) to establish baseline respiration rates of MW, RFB and LGB, and 2) to evaluate the effects of temperature levels and the presence of a food source on the respiration rates.

2. Materials and Methods

2.1. Insect rearing

As an initial disinfestation treatment, brown jasmine rice was packed and sealed in Ziploc® bags and stored at 0±2°C for 1–2 months. After that, the bags were stored at room temperature (30±3°C). Rice bran was disinfested by heat-treating at 80°C for 8 h and then it was stored in aluminum foil at room temperature. Maize weevil, RFB and LGB were obtained from the Postharvest and Processing Research and Development Office, Department of Agriculture, Thailand. MWs and LGBs were reared using the brown jasmine rice. For each species, adult insects were left to lay eggs at room temperature in 270-ml glass jars each of which contained 250 g of brown jasmine rice. There were 500 individuals in each jar. After 3 days, the adult insects were separated from the rice. The rice was then left in the jars at room temperature to allow for the eggs to develop into adults. RFBs were cultured in a similar manner using the rice bran as rearing media. Each rearing jars of RFBs contained 300 g of rice bran. For all three species, respiration rates were measured from newly emerged adult insects which were no more than 1 month old. Also note that the disinfested brown rice was also used as the food source while measuring respiration rates of insects of all species.

2.2. Experimental plan

The experiments in which insect respiration rates were determined with and without the presence of food were planned and conducted separately. For each case, a split-plot experimental design was implemented whereby the main plot was the surrounding temperature (25, 30 and 36°C) and the sub plot was the number of insects (50, 100, and 200

insects). Thus, each experiment (Table 1) consisted of nine combinations of test conditions (i.e., three temperature levels × three numbers of insects). For each species, five sets of adult insects were prepared. Insects in each set were divided into three groups in which the numbers of insects were 50, 100 and 200. Each set was specified as an experimental block (blocks 1–5) and was tested at the same temperature. As an example, for the test condition of no food source at 25°C temperature, the respiration rates of the 50, 100 and 200-insect groups from the same set were measured simultaneously. Each group of insects was placed in a 650 ml plastic container. A 35 cm^2 square hole was cut on the container's lid and covered with a stainless steel mesh. For the experiments with a food source, the insects were placed in the container along with 125 g of brown Jasmine rice. Each plastic container was placed in a glass desiccator along with a non-dispersive infrared CO_2 data logger (CO210, Extech Instrument Corp., Nashua, New Hampshire, USA). The gap between lid and top edge of glass desiccator was sealed with parafilm® to prevent leakage of CO_2 gas. The desiccator was put in a temperature control chamber (locally fabricated) for at least 15 h. The CO_2 data logger recorded the CO_2 gas concentration in the desiccator every 15 s. Note that in a separate preliminary test it was found that brown jasmine rice did not respire (data not shown).

Table 1 Summary of the split-plot experimental design which was implemented for each insect species with and without the presence of food separately.

Temperature (main plot):	25, 30 and 36°C
Number of insects (sub plot):	50, 100 and 200
Insect portion (block):	1, 2, 3, 4 and 5

2.3. Data processing

The CO_2 data loggers recorded CO_2 gas concentrations in units of ppm by volume (part per million by volume) or μl_{CO2} $(l_{air})^{-1}$ (microliter of CO_2 per liter of air). Every recorded data point was subtracted by the initial background CO_2 concentration in the desiccator, yielding CO_2 concentration increases exclusively due to the respiration of insects. In a preliminary experiment, the rate of change of CO_2 concentrations was found to be linear and thus was described by the following equation:

$$c = at \tag{1}$$

where c is the CO_2 gas concentration ($\mu l_{CO2}/l_{air}$), a is the rate of change ($\mu l_{CO2}/l_{air}$ h) and t is elapsed time (h). At the beginning of each experimental replicate, the insects were acclimating to the environment in the desiccator and the CO_2 concentrations during this time were not yet linear. As a result, CO_2 concentrations collected in the first five hours of each replicate were truncated. Next, the truncated CO_2 concentration curve was fitted with equation 1. In order to obtain the respiration rate of one individual insect in units of μl_{CO2}/insect h, the slope of the fitted curve (i.e., rate of change of CO_2 concentrations) was multiplied and divided by the air volume in the desiccator, V_{air} (l) and the total number of insects, respectively. The air volume in the desiccator was calculated as:

$$V_{air} = V_{desiccator} - V_{logger} - V_{container} - V_{jas} - V_{insect} \tag{2}$$

where $V_{desiccator}$ is the air volume inside the desiccator when emptied (l), V_{logger} is the solid volume of the CO_2 data logger (l), $V_{container}$ is the solid volume of the plastic container (l), V_{jas} is the solid volume of brown jasmine rice (l) and V_{insect} is the solid volume of the insects (l). $V_{desiccator}$, $V_{container}$ and V_{jas} were determined by the water replacement method. V_{logger} was determined using the pressure decay rate method described by Chayaprasert et al. (2014). The

shape of all insects were assumed cuboidal and thus the volume of an individual insect could be calculated as width × length × height. For each insect species, the three dimensions of 50 individuals were measured using a Vernier caliper (Mitutoyo Corp., Kawasaki, Japan). The volume of one insect was averaged from these 50 individuals. V_{insect} was the multiplication between the single-insect average volume and the number of insects used in the experiment. For each combination of the insect number and temperature level, the insect respiration rate was calculated as the average value of all respiration rates from the five sets of prepared insects (i.e., experimental blocks 1–5). In order to determine the effect of surrounding temperature on the respiration rate, the average respiration rates were analyzed according to the split-plot experimental design. In order to evaluate the effect of the different numbers of insects on the insect respiration rate measurement, the entire data set was rearranged and reanalyzed using one-way ANOVA.

3. Results and Discussion

Figure 1 illustrates an example of measured CO_2 concentrations from the 100-insect group of block 4 at 30°C for each insect species. All three insect species yielded linear increases in CO_2 concentrations. In this study, when fitted with equation 1, the coefficients of determination (R^2) of only two CO_2 concentration curves were less than 0.9. This showed that insects respire at constant rates given that surrounding temperature does not change. The average respiration rates for all experimental conditions are listed in Table 2. Regardless of surrounding temperatures, all three insect species respired at higher rates when a food source was provided. When averaging across the insect numbers, without the presence of food the respiration rates of MW, LGB and RFB were 2.58–5.47, 1.04–2.23 and 1.02–2.73 μl_{CO2}/insect h, respectively. Similarly, when food was present, the respiration rates increased to 10.15–13.76, 1.77–4.19 and 4.69–11.33 μl_{CO2}/insect h, respectively. This indicated that in the presence of food insects had increased physical activities. According to the split-plot ANOVA results (Table 3), for each insect species and each case of food presence temperature variations had a significant effect on the respiration rates ($P<0.05$). Without a food source, for all insect species the respiration rates increased as the temperature increased from 25 to 36°C. However, in the opposite case the respiration rates of MW and RFB were highest at 30°C. This finding implied that for a CO_2 based grain quality monitoring system to be able to accurately estimate the insect population in a storage structure the temperature inside the structure should be taken into account. In addition, in some cases (i.e., MW and RFB with no food and LGB with food) the different numbers of insects used in the experiments (i.e., 50, 100 and 200) had a significant effect on the respiration rates. In order to determine only the effect of insect numbers, the respiration rate data were regrouped and one-way ANOVA was performed for each combination of temperature, insect species and food presence (Table 4). With one-way ANOVA, the insect numbers significantly affected the respiration measurement only for LGB with food at 36°C ($P=0.02$). When applying a Duncan's multiple range test to this specific case, the respiration rate of the 50-insect group was classified as significantly different from those of the 100- and 200-insect groups. Thus, with the technique used for measuring insect respiration rates in the present study a sample size of at least 100 insects is recommended for a single measurement.

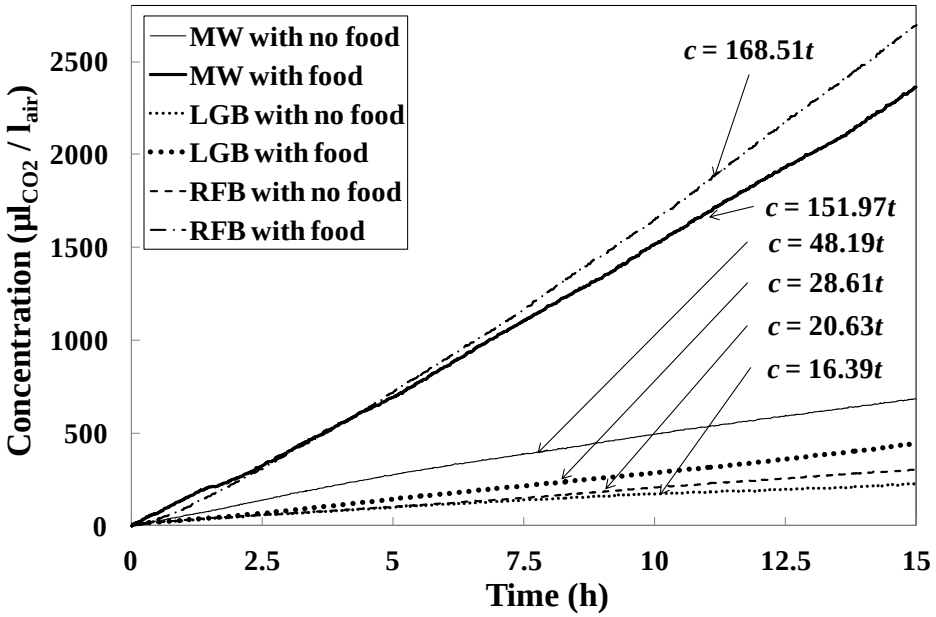

Figure 1 CO_2 concentrations curves collected from the 100-insect group of block 4 at 30°C.

Table 2 Respiration rates (average±SD, n=5) of three insects species tested in this study.

Conditions	Number of insects	Respiration rate of insect (µl$_{CO2}$/insect h)								
		MW			LGB			RFB		
		25°C	30°C	36°C	25°C	30°C	36°C	25°C	30°C	36°C
No food	50	2.11 ±0.27	3.45 ±0.47	5.62 ±0.57	1.00 ±0.16	1.58 ±0.31	2.18 ±0.30	0.87 ±0.24	1.65 ±0.30	2.66 ±0.70
	100	2.66 ±0.56	3.76 ±0.22	5.50 ±0.54	1.01 ±0.11	1.54 ±0.24	2.15 ±0.29	1.07 ±0.11	1.86 ±0.39	2.72 ±0.62
	200	2.96 ±0.63	3.64 ±0.25	5.28 ±0.35	1.10 ±0.05	1.62 ±0.11	2.36 ±0.22	1.13 ±0.14	2.05 ±0.35	2.81 ±0.65
	Avg.	2.58 ±0.43	3.62 ±1.06	5.47 ±0.17	1.04 ±0.06	1.58 ±0.04	2.23 ±0.12	1.02 ±0.14	1.86 ±0.20	2.73 ±0.07
With food	50	11.00 ±1.93	13.42 ±2.65	11.10 ±1.20	1.58 ±0.21	2.96 ±0.46	4.96 ±0.88	3.96 ±2.02	12.16 ±2.74	9.87 ±2.10
	100	11.95 ±1.21	14.13 ±1.43	9.57 ±1.22	1.96 ±0.26	2.97 ±0.34	3.93 ±0.35	4.59 ±2.53	11.76 ±3.74	9.87 ±2.25
	200	12.91 ±0.50	13.72 ±1.29	9.79 ±1.13	1.77 ±0.22	2.67 ±0.24	3.69 ±0.50	5.54 ±2.15	10.09 ±1.82	9.94 ±1.87
	Avg.	11.95 ±0.95	13.76 ±0.36	10.15 ±0.83	1.77 ±0.19	2.87 ±0.17	4.19 ±0.68	4.69 ±0.79	11.33 ±1.10	9.89 ±0.04

Table 3 Summary of the ANOVA results according to the split-plot experimental design for each insect species and each case of food presence.

Source of variation	P-value					
	MW		LGB		RFB	
	No food	With food	No food	With food	No food	With food
Main plot:						
Temp.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Block (insect portion)	0.98	0.39	0.67	0.64	0.27	0.11
Sub-plot:						
Number of insects	<0.01	0.71	0.07	<0.01	<0.01	0.93
Temp. × Number of insects	<0.01	0.02	0.78	<0.01	0.30	0.14

Table 4 One-way ANOVA results indicating the effect of insect numbers on the respiration rates.

Temp. (°C)	P-value					
	MW		LGB		RFB	
	No food	With food	No food	With food	No food	With food
25	0.06	0.12	0.34	0.07	0.07	0.55
30	0.35	0.84	0.87	0.34	0.23	0.50
36	0.57	0.13	0.44	0.02	0.94	0.99

4. Conclusions

The respiration rates of MW, RFB and LGB were determined with and without the presence of food at three constant surrounding temperatures. Concentrations of the CO_2 gas generated by these stored product insects were measured by non-dispersive infrared CO_2 data loggers in sealed glass desiccators. In all cases, measured CO_2 concentrations increased linearly, indicating that the insects respired at constant rates. Regardless of surrounding temperatures, all three insect species respired at higher rates when a food source was provided. Surrounding temperature variations had a significant effect on the respiration rates. Without the presence of food the average respiration rates of MW, LGB and RFB were 2.58–5.47, 1.04–2.23 and 1.02–2.73 μl_{CO2}/insect h, respectively. Similarly, when food was present, the respiration rates increased to 10.15–13.76, 1.77–4.19 and 4.69–11.33 μl_{CO2}/insect h, respectively. Without a food source, the respiration rates of all insect species increased as the temperature increased from 25 to 36°C. However, in the opposite case the respiration rates of MW and RFB were highest at 30°C. This finding implied that when estimating the insect population in a storage system based on CO_2 gas concentrations, the temperature inside the structure should be incorporated into the estimation. With the technique used for measuring insect respiration rates in this study, a sample size of at least 100 insects is recommended for a single measurement. From the perspective of grain quality management, this study provided important baseline data and experimental protocol for developing decision support tools based on quantitative insect population.

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References

Chayaprasert, W., Chantalak, A., Sukcharoen, A., 2014. Porosity measurement of granular materials by comparisons of air pressure decay rates. Transactions of the ASABE 57, 1431-1440.

Emekci, M., Navarro, S., Donahaye, E., Rindner, M., Azrieli, A., 2002. Respiration of *Tribolium castaneum* (Herbst) at reduced oxygen concentrations. Journal of Stored Products Research 38, 413-425.

Emekci, M., Navarro, S., Donahaye, E., Rindner, M., Azrieli, A., 2004. Respiration of *Rhyzopertha dominica* (F.) at reduced oxygen concentrations. Journal of Stored Products Research 40, 27-38.

Fonseca, S.C., Oliveira, F.A.R., Brecht, J.K., 2002. Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. Journal of Food Engineering 52, 99-119.

Guedes, R.N.C., Oliveira, E.E., Guedes, N.M.P., Ribeiro, B., Serrao, J.E., 2006. Cost and mitigation of insecticide resistance in the maize weevil, *Sitophilus zeamais*. Physiological Entomology 31, 30-38.

Hubert, J., Pekár, R., Nervorná, M., Šustr, V., 2010. Temperature preference and respiration of acaridid mites. Journal of Economic Entomology 103, 2249-2257.

Lu, B., Ren, Y., Du, Y.-z., Fu, Y., Gu, J., 2009. Effect of ozone on respiration of adult *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.). Journal of Insect Physiology 55, 885-889.

Neven, L.G., 1998. Respiratory response of fifth-instar codling moth (Lepidoptera: Tortricidae) to rapidly changing temperatures. Journal of Economic Entomology 91, 302-308.