Study on the mortality of the stored-grain insects adults in different concentrations of low oxygen

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Abstract

During this research, adults of *Liposcelis bostrychophila* Badonnel, *Oryzaephilus surinamensis* (Linnaeus), *Tribolium confusum* Jacquelin du Val, two strains of *Tribolium castaneum* (Herbst) (from Zhongshan and Yiyang), *Sitophilus oryzae* (Linnaeus) and *Sitophilus zeamais* Motschulsky were first kept in various low oxygen atmospheres whose concentrations were 0%, 1% and 2% for different exposure times, then in the normal atmosphere. The data and trend of mortality changes accompanied by the low oxygen concentration and exposure time were gained after this research. Results indicate that there were obvious inter-specific differences among the test insects in the sensitivity to low oxygen atmosphere, and the sensitivity declined as follows: *L. bostrychophila*, *O. surinamensis*, *T. confusum*, *T. castaneum*, *S. oryza*e and *S. zeamais*. The insects of closely related species presented diverse responses to the low oxygen stress. *Tribolium confusum* and *T. castaneum* showed a very dissimilar sensitivity to the low oxygen atmosphere, but there was no obvious difference between *S. oryzae* and *S. zeamais*. A fast lethal effect on the adults of *L. bostrychophila*, *O. surinamensis*, *T. confusum* and *T. castaneum* was observed when exposed to 0% oxygen. At 1% and 2% oxygen atmosphere, the mortality level of *T. castaneum* exceeded 60% and 80%, respectively. The lethal effect of 2% was more efficient than 1% oxygen, which was the same as *S. oryzae* and S. *zeamais* at 2% oxygen atmospheres. The mortality of *S. oryzae* and S. *zeamais* at 1% and 2% oxygen concentration was higher than at 0%, and the mortality of these two insects might be higher under the condition of a small amount of oxygen. At 1% and 2% oxygen atmosphere, the growth trend of the cumulative mortality of *T. confusum* and *T. castaneum* generally conformed to the classical model of the Logistic formula which indicated the response of insect pests when applying common insecticide. These results are useful to extend the control technology of stored-grain insects with low oxygen universally.

Keywords: Low oxygen; Stored-grain insect; Mortality; Treatment duration

1. Introduction

As a green control technology of stored-grain insects, the low oxygen disinfestation technology has been more and more popular with many companies in China. In order to get a scientific practical application of it, the worldwide studies have focused on the lethal effect of different low oxygen concentrations to kinds of stored-grain insects. As reported, atmosphere where the oxygen content was less than 1%, the lethal time of *O. surinamensis, R. dominica* and *S. oryzae* was more than 1 d, 4 d and 14 d respectively, and that of *T. confusum* and *T. castaneum* was more than 7 d at 20ºC to 29ºC (Banks and Fields, 1994). The stored-grain insects, such as *R. dominica, S. granarius* and *S. oryza*e adults were more tolerant than *C. ferrugineus*, *O. surinamensis* and *T. castaneum* at various low oxygen concentrations (Krishnamurthy et al., 1986; Conyers and Bell, 1996). In China, the disinfestation effect of high-purity nitrogen atmosphere (almost anaerobic) to common stored-grain insect adults was studied (Zhang et al., 2007.). In view of many studies, Annis (1987) made many useful suggestions on the relationship between the oxygen content and exposure time in the application of low oxygen disinfestation technology.

In recent years, with the gradually increasing demand of the green grain storage, there was a large scale application of controlled atmosphere storage technology in China. To reduce the costs of grain storage by the CO_2 controlled atmosphere, the low oxygen storage technology was studied, the control of stored-grain insect with low oxygen technology and the green low oxygen grain storage technology were carried out, and until now some good effects have been achieved in China. Until May 2009 in China, there were more than 20 national grain depots which conducted the application of “Nitrogen-rich and Low-oxygen

Grain Storage Technology" (NLGST), a great progress in green grain storage technology has been made in China. In the present study, adults of *L. bostrychophila*, *O. surinamensis*, *T. confusum*, *T. castaneum*, *S. oryzae* and *S. zemais* were exposed to different low oxygen concentrations and exposure times, then they were kept at normal atmosphere. The mortality of all these pest species was analyzed, with the aim to provide more theoretical support for the promotion of NLGST.

2. Materials and methods

2.1. Culture of the test insects

Sitophilus zeamais and *S. oryzae* sampled from Guangzhou, Guangdong Province were reared on wheat of moisture content 14 ± 2%; *T. confusum* form Puyang, Henan Province, and two *T. castaneum* strains form Yiyang, Hunan Province and Zhongshan, Guangdong Province were reared on a mixture ($w_{\text{whole wheat flour}}/w_{\text{yeast}}$ = 19/1); *O. surinamensis* form Nanyang, Henan Province was reared on a mixture of feeds ($w_{\text{oat}}/w_{\text{whole wheat flour}}/w_{\text{yeast}}$ = 6/3/1); *L. bostrychophila* form Xinshagang, Guangdong Province was reared on a mixture of feeds ($w_{\text{whole wheat flour}}/w_{\text{yeast}}/w_{\text{milk powder}}$ = 1/1/1) passed a screen by 187.5 μm. All of them were maintained at 30 ± 1ºC (75 ± 5 % r.h.).

2.2.1. The experimental equipment

The experimental equipment consisted of the gas source, flux control device, experiment container and pipelines(Zhang et al., 2007). To ensure purity and stability of the gas, we chose high-purity N_2 (GB 8980-88) and medical O_2 (GB 8982-88) as the gas source. The glass desiccators were used for treating test insects with condition of low oxygen atmosphere, and desiccator lids were sealed gastight using Vaseline.

2.2.2. The assessment

Adults of 7-10 d old were chosen as test insects. Batches of 50 insects were placed in each culture dish with a diameter of 75 mm, whose wall was coated with teflon, and the bottom was covered with filter paper. Furthermore, crushed wheat of 1 g/dish was added for *S. oryza*e and *S. zeamais*. *T. castaneum*, *T. confusum*, *O. surinamensis* were kept with 0.1 g/dish while for *L. bostrychophila* was kept with of 0.001 g/dish.

All experiments were repeated three times, and the control group was exposed to the normal atmosphere for the same exposure time as the treatments. All experiment groups were maintained at 30 ± 1ºC (75 ± 5 % r.h.). In order to mitigate their discomfort caused by the container or else, the test insects were placed into the desiccator 24 h before the experiment. After that, according to Table 1, 2 and 3, the test insects were treated for different exposure times at 0%, 1% and 2% oxygen.

Table 1 Exposure time (h) of different test insects in the oxygen concentration of 0% at 30 ± 1°C and 75 ± 1% r.h.

Test insects strains	**Experiment group**						
	A	**B**	**C**	**D**	**E**	**F**	**G**
L. bostrychophila	2	3	4	5	6	7	8
O. surinamensis	3	4	5	6	7	8	9
T. castaneum (Zhongshan)	11	13	15	17	19	21	23
T. castaneum (Yiyang)	10	11	12	13	14	15	16
T. confusum	10	11	12	13	14	15	16
S. oryzae	25	27	29	31	33	35	37
S. zeamais	25	27	29	31	33	35	37

Table 2 Exposure time(h) of different test insects in the oxygen concentration of 1% at 30 ± 1°C and 75 ± 1% r.h.

Test insects strains	Experiment group						
	A	B	C	D	E	F	G
L. bostrychophila	12	14	16	18	20	22	24
O. surinamensis	12	18	24	30	36	42	48
T. castaneum (Zhongshan)	12	18	24	30	36	42	48
T. castaneum (Yiyang)	12	18	24	30	36	42	48
T. confusum	12	18	24	30	36	42	48
S. oryzae	12	18	24	30	36	42	48
S. zeamais	12	18	24	30	36	42	48

Table 3 Exposure time(h) of different test insects in the oxygen concentration of 2% at 30 ± 1°C and 75 ± 1% r.h.

Test insects strains	Experiment group						
	A	B	C	D	E	F	G
L. bostrychophila	23	26	29	32	35	38	41
O. surinamensis	12	24	36	48	60	72	84
T. castaneum (Zhongshan)	12	24	36	48	60	72	84
T. castaneum (Yiyang)	12	24	36	48	60	72	84
T. confusum	12	24	36	48	60	72	84
S. oryzae	12	24	36	48	60	72	84
S. zeamais	12	24	36	48	60	72	84

After treatment in the low oxygen atmosphere, the culture dishes and the test insects were removed from the desiccators together and ventilated for 20-30 min in the normal atmosphere. After that, all groups were maintained in the normal atmosphere at 30 ± 1°C (75 ± 1% r.h.), and added the feeds properly. The mortality changes were daily recorded until they were stable.

3. Results and discussion

3.1. The cumulative mortality and changes of L. bostrychophila adults in three different low oxygen concentrations

The mortality curves of *L. bostrychophila* in the oxygen atmosphere of 0%, 1% and 2% are shown in Figure 1. For 0% treatment, the mortality of *L. bostrychophila* increased fast, and reached 100% after 6 h. In 1% oxygen concentration, it needed 24 h to reach the mortality of 100%. And for 2% treatment group, it needed 48 h. The results indicated that, the lower the oxygen contents the shorter the lethal time.

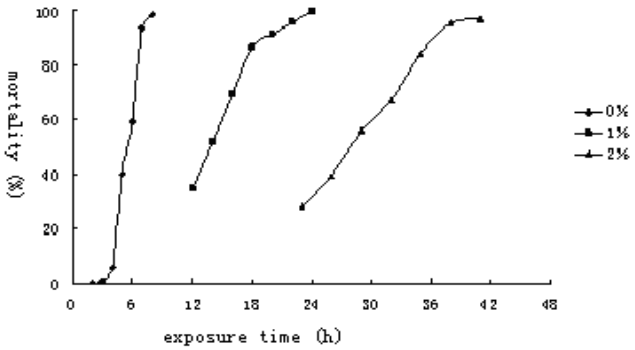

Figure 1 The mortality curve of *L. bostrychophila* adults in three different low oxygen concentrations.

Despite the difference of more than 10 h in the lethal time, the mortality rate of 1% and 2% samples increased almost simultaneously within the mortality of 30%-90%. These results suggest that adults exposed to the above two treatments might have the same sensitivity to the low oxygen. The mortality raise to 85% or more was gradual and slow, which indicated that there was a group of individuals with higher tolerance, and a slower change of the sensitivity to low oxygen than the earlier dead insects. But the relevant reason still needed to be further studied.

As Figure1 showed, the mortality curves of three different concentrations of oxygen separated clearly. These results suggest that *L. bostrychophila* might be very sensitive to changes of the low oxygen concentration, and slight change of oxygen content could greatly affect its lethal impact.

3.2. The mortality curve of O. surinamensis adults in three different low oxygen concentrations

The mortality curve of *O. surinamensis* in the oxygen content of 0%, 1% and 2% was shown in Figure 2. At 0% oxygen, the mortality of *O. surinamensis* increased rapidly, and reached 100% after 6 h. At 1% oxygen, the mortality after 24 h was about 92%. And at 2% oxygen, the mortality was 60% after 24 h. The results showed that, with the reduction of the oxygen content, the duration to the mortality of 100% was significantly reduced. For the treatment of 1% and 2% oxygen, the mortality increased much more slowly than 0%. These two treatments responded similarly at exposure to 12 h, and at longer exposure time the mortality response separated gradually, and the treatment of 1% reached to the mortality of 100% earlier.

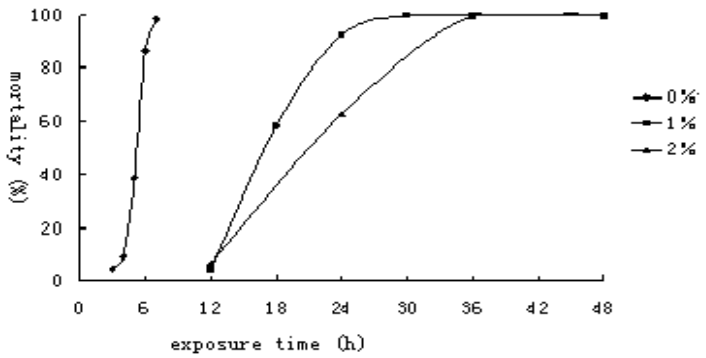

Figure 2 The mortality curves of *O. surinamensis* in three different low oxygen concentrations.

3.3. The mortality of T. confusum in three different treatments of low oxygen

The mortality curves of *T. confusum* at the low oxygen concentration of 0%, 1% and 2% were shown in Figure 3. At the oxygen content of 0%, the morality of *T. confusum* increased within short exposure times, and attained 100% after 16 h. The mortality at 1% and 2% oxygen was less than 20% at shorter exposures than 24 h. Then at exposure of 48 h, the mortality of the test insects reached close to 100%. However, the response of the insects differed obviously to these two low oxygen treatments. After the treatment of 1% oxygen for 36 h, the mortality reached as high as 95%. At 2% oxygen, the mortality gradually increased, reached only 40% after 36 h, and then prolonging the exposure to 48 h, resulted in up to 95% mortality. In addition, for the 1% oxygen, the cumulative morality response of the pest followed the trend of “S” type curve.

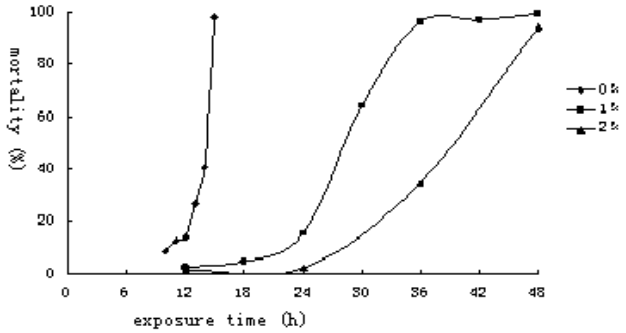

Figure 3 The mortality curves of *T. confusum* at three different treatment of low oxygen.

3.4. The mortality of T. castaneum (Yiyang strains) in three different treatments of low oxygen

The mortality curve of *T. castaneum* (Yiyang) in the low oxygen atmosphere of 0, 1 and 2% under experimental conditions were presented as Figure 4. For the oxygen content of 0%, the morality of the pests increased rapidly, and reached the peak 100% after 12 h. At 1 and 2% oxygen, the mortality raise tardily, and no mortality was observed before 18 h exposure. Then with the extension of the exposure time, the mortality at the two oxygen concentrations began to increase, and after 48 h the two mortality curves reached up to 80 and 100%, respectively. Under the test conditions, the mortality curves of 1 and 2% began from the same starting point, and then gradually increased with near linearity, and there was an incorporated trend with a crossover at 36 h, and the mortality of 2% oxygen exceeded that of 1%.

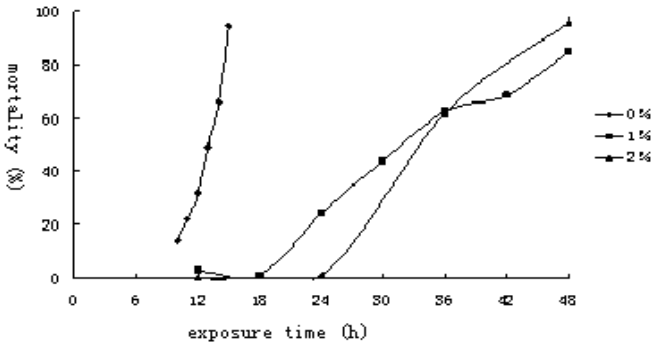

Figure 4 The mortality curves of *T. castaneum* (Yiyang) at three different treatment of low oxygen.

3.5. The mortality of T. castaneum (Zhongshan) in three different treatments of low oxygen

The mortality curves of *T. castaneum* (Zhongshan) in the oxygen content of 0%, 1% and 2% were shown in Fig. 5. For the treatment of 0%, the mortality of the pests increased fast, and went up to 100% after 16 h. The mortality curves of 1% and 2% groups resembled *T. castaneum* (Yiyang). After exposure to 24 h, there was almost no change in the mortality level. When prolonged the exposure time to 34 h, it was found that both of the two oxygen concentrations caused to increased mortality, and reached more than 80% and the two curves were very close to each other. Insect mortality at 1% oxygen ascended slowly with a stable level at 80%, but the 2% oxygen went up to 99% mortality rapidly from 80% in 18 h, and the effective time lasted 48 h, whose mortality of 2% oxygen was strikingly exceeded the 1% oxygen. In addition, for 1% and 2% oxygen, the cumulative mortality of the test insects increased and followed the shape of "S".

The sensitivity to low oxygen of *T. castaneum* (Yiyang) and of *T. castaneum* (Zhongshan) showed many similarities to hypoxia condition. It was found that the mortality was very low after treatment for 18 or 24 h at 1 and 2% oxygen, accelerated when extended to 36 h, crossed at 60 and 80%, afterwards exposure to 2% oxygen resulted in higher mortality than at 1% oxygen. Mortality of *T. castaneum* (Yiyang) reached close to 100% after 48 h (Fig. 4); while the mortality of *T. castaneum* (Zhongshan) changed slowly (Figure 5). The mortality remained steady 80% at 1% oxygen, however, at 2% oxygen after 48 h reached 95%. Furthermore, compared with *T. confusum* (Fig. 3), there was no crossing for 1 and 2% groups, which suggested that there was obvious difference between *T. confusum* (Fig. 3) and *T. castaneum* (Fig. 4 and 5) in the sensitivity to the hypoxia atmosphere, and the same strains had a similar sensitivity, and the reason needed to be deeply analyzed.

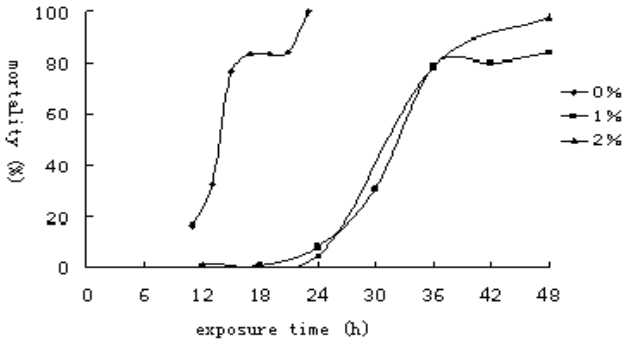

Figure 5 The mortality curves of *T. castaneum* (Zhongshan) at three different treatment of low oxygen.

3.6. The mortality of S. oryzae in three different treatments of hypoxia

The mortality curves of *S. oryza*e in the oxygen content of 0%, 1% and 2% were shown in Figure 6. The results showed that the mortality of the three test groups went up immediately. *Sitophilus oryza*e was less sensitive to hypoxia at 0% oxygen than at 1 and 2%, in which group it needed a longer exposure time for mortality. At 0% oxygen, the mortality was only around 75%, however, the mortality at 1 and 2% oxygen was 82 and 86%, respectively for the same exposure times. That is to say, under the condition of 1 and 2% oxygen, *S. oryza*e were more sensitive than at 0% oxygen within the mortality range of 10 - 80%.

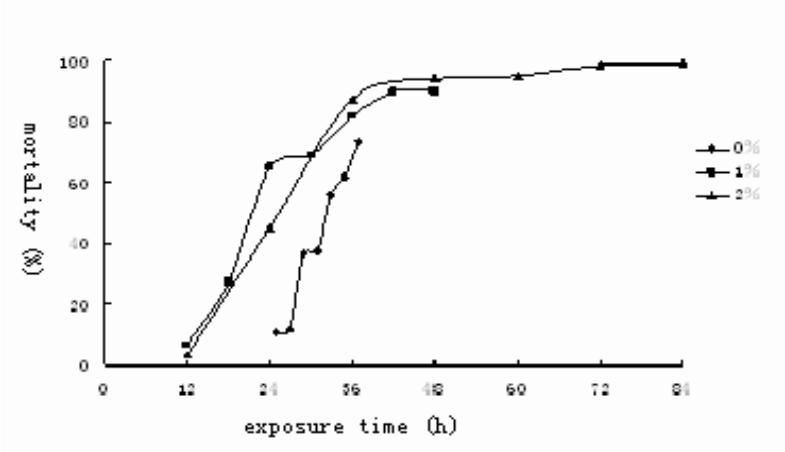

Figure 6 The mortality curves of *S. oryza*e at three different treatment of low oxygen.

There was a merged trend in the mortality curves at 1 and 2% oxygen. Moreover, the growth became very slow after the mortality of more than 90%. For the mortality at 2% oxygen, the exposure time to achieve from 86% to nearly 99% mortality lasted for 44 h from the 36 h to the 80 h. This indicates that some individuals were very tolerant to the hypoxia atmosphere, and we should pay attention to these high-tolerant individuals to monitor that whether *S. oryza*e can be used as the tolerant insect species to the hypoxia condition.

3.7. The mortality of S. zeamais in three different treatments of low oxygen

The mortality curves of *S. zeamais* in the oxygen content of 0, 1 and 2% were shown in Figure 7, which were similar to *S. oryza*e. For the treatment of 0%, the mortality was less than 75% after 36 h, which was slightly higher than 1, and 2% oxygen that reached 87% mortality. At 1 and 2% oxygen, the mortality curve fit well together from 0% to 60%, then began to separate after that. For 2% oxygen, the duration in which the mortality increased from 87 to 99% lasted for 48 h from the 36 h to the 84 h. These results suggested that similar to *S. oryza*e, there were also *S. zeamais* individuals tolerant to low oxygen atmosphere.

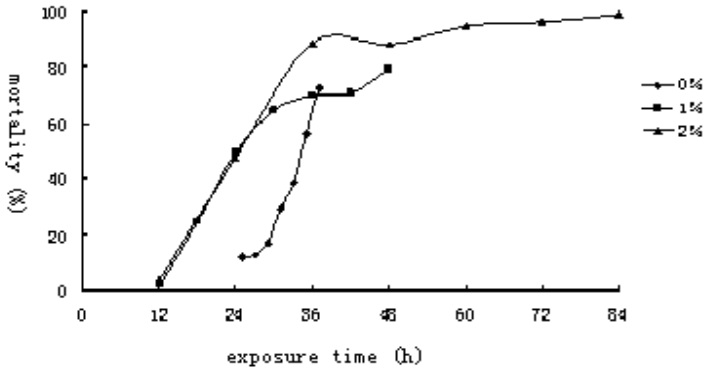

Figure 7 The mortality curves of *S. zeamais* at three different treatments of low oxygen.

The mortality curves of *S. zeamais* and *S. oryza*e to the low oxygen treatment presented similarities in response (Fig. 6 and 7), which was different from *T. confusum* and *T. castaneum*. In addition, the mortality at 1 and 2% oxygen, was higher than 0% oxygen. That is to say, the tolerance of *S. oryza*e and *S. zeamais* to the oxygen concentration at 0% was stronger and more specific, which is attributed to the spiracular closing mechanism of the adult beetles. At the lowest oxygen concentration the beetles strongly close their spiracles and in this way they prevent water loss, whereas at higher oxygen levels 1 and 2% they enable ventilation through their spiracles and death is caused due to desiccation (Navarro, 1978)

4. Conclusions

1. The sensitivity of five storage insect pests to the hypoxia treatment of 0, 1 and 2% oxygen were observed in declining order as follows: *L. bostrychophila*, *O. surinamensis*, *T. confusum*, *T. castaneum*, *S. oryza*e and *S. zeamais*, which presented a significant diversity.
2. The trend of the cumulative mortality of *T. confusum* and *T. castaneum* at 1 and 2% oxygen atmosphere generally conformed to the classical model of Logistic formula which indicated the mortality growth of pests when applying common insecticide.
3. 0% oxygen concentration has a quick lethal effect on the adults of *L. bostrychophila*, *O. surinamensis*, *T. confusum*, and *T. castaneum*.

4. The adults of closely related species showed a diverse tendency to the hypoxia stress. The mortality response of *T. confusum*, *T. castaneum* varied widely; however, *S. oryza*e and *S. zeamais* responded almost in identical trend.
5. In 1 and 2% oxygen concentration atmosphere, the mortality curves of crossed at 60 and 80% mortality, and the lethal effect of 2% oxygen was more efficient than that of 1%. Furthermore, under the same conditions *S. oryza*e and *S. zeamais responded similarly*.
6. The mortality of *S. oryzae* and *S. zeamais* in 1 and 2% oxygen concentration was higher than 0% oxygen, and in other words the mortality of these two pests might be higher in the presence of a small amount of oxygen

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