

Tao Z, Shiwei L, Yanyu L, Yang C, Guangtao L, Yongkui C, Zihai Z (2012) Mortality of four stages of *Lasioderma serricorne* (Fabricius) exposed to low oxygen. In: Navarro S, Banks HJ, Jayas DS, Bell CH, Noyes RT, Ferizli AG, Emekci M, Isikber AA, Alagusundaram K, [Eds.] Proc 9th. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products, Antalya, Turkey. 15 – 19 October 2012, ARBER Professional Congress Services, Turkey pp: 17-25

## MORTALITY OF FOUR STAGES OF *LASIODERMA SERRICORNE* (FABRICIUS) EXPOSED TO LOW OXYGEN

Zhang Tao<sup>1</sup>, Liu Shiwei<sup>2</sup>, Li Yanyu<sup>1</sup>, Cao Yang<sup>1</sup>\*, Li Guangtao<sup>1</sup>, Cui Yongkui<sup>2</sup>, Zhang Zihai<sup>2</sup>

<sup>1</sup> Academy of State Administration of Grain, №11 Baiwanzhuang Street, Beijing, 100037, P.R. China, Tel: 86-10-68623665. Fax: 86-10-58523599

<sup>2</sup> Hunan Hua Wang Fumigation Technology Co., Ltd, №523, East Laodong Road, Yuhua District, Changsha 410014, P.R. China

\*Corresponding author's e-mail: [cy@chinagrain.org](mailto:cy@chinagrain.org)

### ABSTRACT

Eggs, lava, pupa, and adults of *Lasioderma serricorne* (Fabricius) were exposed at different temperatures in 2% oxygen, 98% nitrogen, and 75% r.h. using the FAO recommended assay method. Probit analysis was used to determine the LT<sub>99</sub> values. The results indicate that the 99% lethal time (LT<sub>99</sub>), (95% Fiducial Limits) of the eggs were for 12.65 d (7.98~73.27), 9.40 d (5.70~41.82), 7.58 d (3.74~8180.93), 5.08 d (4.48~5.97), 2.86 d (2.39~3.81) at 15°C., 20°C., 25°C., 30°C., and 35°C, respectively in 2% oxygen, 98% nitrogen, 75% r.h.. The LT<sub>99</sub> for eggs was increased by 0.48 d with the reduction of temperature by each 1°C. At 30°C and the same conditions, the LT<sub>99</sub> of the larvae and the pupae were 9.62 d (8.86~10.74), and 8.07 d (7.22~9.58), respectively. At 30°C. and 35°C., the LT<sub>99</sub> for adults was 21.93 d (14.28~83.47), and 13.43 d (8.32~40.34), respectively. Based on the comparison of the LT<sub>50</sub> values of the four development stages of *L. serricorne* the sensitivity level to low oxygen in decreasing order was: eggs, adults, pupa, and lava. Based on the comparison of the LT<sub>99</sub> values the sequence of sensitivity in decreasing order was: eggs, pupa, lava, and adults.

**Key words:** *Lasioderma serricorne*, low oxygen, lethal time

### INTRODUCTION

*Lasioderma serricorne* (Fabricius) belongs to the family of Coleoptera; Anobiidae and is harmful to the tobacco, cigarettes and cigar products in storage (Jizhen et al., 2006), causing remarkable value loss to tobacco industry. *L. serricorne* is known as the world's number one pests of stored tobacco (Jianhua et al., 2010). *L. serricorne* has a wide range of diets like food grains, teas, beans, dried jujube, oil products, plant and animal specimen, cocoa bean, leather, vine and bamboo products (Ryan , 1995; Guangjun et al., 2000). *L. serricorne* can be rolled

into the cigarette and feed on tobacco, damage the outside paper, meanwhile, the fragment of its corpses and faeces also pollute the tobacco products. (Arbogast et al.,2002; Xinwen et al., 1995; Fangxiao et al., 2000).

The growth and development impact of harmful pests to stored bulk products at low oxygen have been of common interest in China and other countries. The adult lethal time of *Oryzaephilus surinamensis* L. is above 1 d at <1% oxygen and that of *Rhizopertha dominica* (Fabricius) above 4 d, *Sitophilus oryzae* (L.) above 14 d, more than 7 d for both *Tribolium castaneum* (Herbst) and *Tribolium confusum* Jacquelin du Val (Burton, 1998). Resistance in different low oxygen environments of adult internal feeders including *R. dominica*, *Sitophilus granarius* L. and *S. oryzae* is generally higher than that of *Cryptolestes ferrugineus* (Stephens), *O. surinamensis* and *T. castaneum* which are external feeders (Conyers et al.,1997; Krishnamurthy et al., 1986). Since studies on lethal time of four stages *L. serricorne* in low oxygen scarce, the present work aimed at investigating the sensitivity of eggs, larva, pupa, and adults of *L. serricorne* exposed to low oxygen.

## MATERIALS AND METHODS

### 1.1 Test insect:

*L. serricorne* was provided by the Stored Grain and Oil Research Laboratory of the Academy of State Administration of Grain. Insects were incubated at  $30\pm 1^{\circ}\text{C}$ ,  $75\%\pm 5\%$  r.h., and reared on whole wheat flour containing 5% yeast.

### 1.2 Test gas

High purified nitrogen, 99.999%, produced by Beijing Bei Temperature Gas Factory. High purified oxygen, 99.99%, produced by Beijing Bei Temperature Gas Factory was used in the present experiments.

### 1.3 Equipments

#### 1.3.1 Main equipment and apparatus:

Fume cupboard, constant temperature incubator (Binder), Orsat gas analyzer, gas flow-meter, rapid oxygen indicator, U-tube pressure meter, electronic drying oven (Shanghai Sumsung Experimental Instrument Co., Ltd), stereomicroscope (Beijing Fuka Keyi Science and Technology Co., LTD), special vacuum dryer, and common dryer were used for the experiments.

#### 1.3.2 Equipment combination:

Equipment combination shown in Fig. 1.

#### 1.3.3 Testing desiccator gas tightness:

The special desiccator was first washed with deionized water and dried, then vaseline was applied to all connections to ensure gastightness. An U-tube pressure meter was used to measure the vacuum desiccator to maintain about 1000 Pa at constant temperature and pressure environment. The pressure value was recorded after it was stabilized in the desiccator. If the pressure remained the same after 24 h, the special desiccator was used in the experiments.

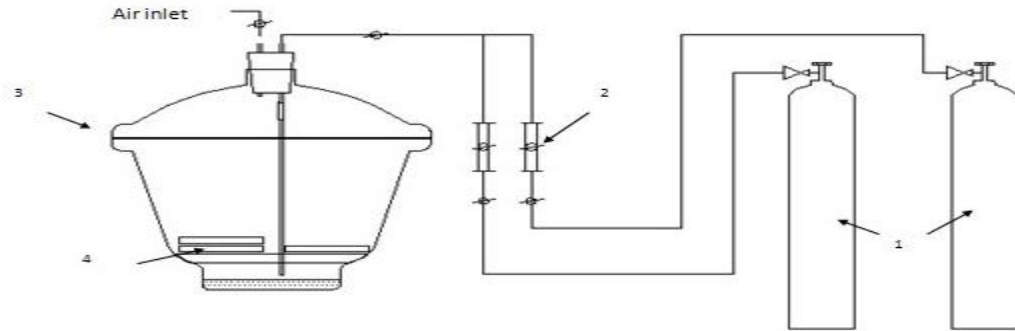


Fig. 1- Diagram of equipment used for exposure of *Lasioderma serricorne* (Fabricius) to low oxygen atmospheres (1= nitrogen and oxygen source; 2= gas flow control gauge; 3= desiccator; 4= insects).

#### 1.3.4 Oxygen content adjustment:

The desired oxygen concentration was obtained by purging the desiccator with nitrogen, and measuring the gas mixture using the Orsat gas analyzer.

#### 1.3.5 Temperature and humidity control:

A temperature controlled incubator (German Binder) was used in which the equilibrium relative humidity was adjusted using saturated saline solutions placed at the bottom of the incubator.

### 1.4 Test method

#### 1.4.1 Treatment of eggs

An egg holding board was used to hold 1 d old 20 eggs which were evenly spread on a slide facing with double-sided adhesive 20 mm×20 mm.. Three in a row of such egg holding boards were glued on a slide for exposure to low oxygen environment; 3 egg holding boards were kept untreated served as control. After treatment the treated egg holding boards were incubated until larva emergence or mortality..

#### 1.4.2 Treatment of larvae

On the wall of a transparent plastic pipe which is 15 mm in diameter and 50 mm long, 50 small holes were pierced. *L. serricorne* culture media was placed in a cage to contain 30 larvae of same age and size and exposed to low oxygen, 3 of such cages were left untreated as control. After treatment, the treated larvae in the cages were incubated and daily observations were made until larvae were dead or turn to pupa.

#### 1.4.3 Treatment of pupae

Culture media of *L. serricorne* together with 30 larva of same age and size were placed in the larva containing cages and kept at 30°C and 75% r.h. for about a week for pupation. Three cages containing the pupae were exposed in a group to low oxygen, 3 untreated cages served as control. After treatment, the treated pupae in the cages were incubated and daily observations were made until pupae were dead or adult emergence.

#### 1.4.4 Treatment of adults

A small piece of tobacco leaf of 14% moisture content together with 30 of 2 d old adults after

emergence were placed in a cage for exposure to low oxygen, 3 untreated cages were kept as control. After treatment, the treated cages were incubated for 24 h to observe their survival under the stereomicroscope. Those adults that did not show any response to touching using a brush were considered as dead.

### 1.5 Statistics

PROC PROBIT method was used to compute the regression line and calculate half lethal time  $LT_{50}$  and 99% lethal time  $LT_{99}$  at 95% Fiducial Limits, b value and its standard deviation. Test of  $\mu$  and t test were carried out on different four development stages to compare  $LT_{50}$  and b values for each stage.

## RESULTS AND DISCUSSION

### 2.1 Effect of low oxygen on *L. serricornis* eggs

Egg mortality of *L. serricornis* exposed to 2% oxygen at 75% r.h. was significantly different at the tested temperatures of 15-35°C. Table 1 shows that temperature plays a significant role on the killing effect of *L. serricornis* eggs exposed to 2% oxygen. The higher was the temperature, the higher was the mortality of eggs. *L. serricornis* eggs were more resistant at the lower temperature region, 99% mortality could be achieved after 10 days of exposure to 2% oxygen at 20°C. and above. Therefore, for the control of *L. serricornis* eggs exposed to 2% oxygen, the temperature should be above 25°C, response of *L. serricornis* eggs 2% oxygen and 75% r.h. was linear to temperature decrease by each 1°C the  $LT_{99}$  increased by 0.48 d (Fig. 2).

Table 1. Mortality of *L. serricornis* eggs exposed to 2% oxygen at 75% r.h. and 15-35°C

time (d)	temperature (°C)				
	15	20	25	30	35
0	5.00±2.89	16.67±1.67	6.10±1.10	11.67±4.41	13.33±1.67
1	\	25.00±5.78	46.67±9.28	21.67±1.67	58.33±13.02
2	20.00±5.00	55.00±12.58	66.67±3.33	56.67±16.91	93.33±1.67
3	\	86.67±4.41	88.33±6.01	90.00±2.89	100.00±0.00
4	40.00±5.78	85.00±0.00	96.67±1.67	96.67±3.33	100.00±0.00
5	\	88.33±3.33	\	100.00±0.00	100.00±0.00
6	76.67±3.33	\	\	\	\
8	96.67±1.67	\	\	\	\
10	100.00±0.00	\	\	\	\
Reg. equation	Y=4.60 lg(x) -2.74	Y=3.56 lg(x) -1.14	Y=2.96 lg(x) -0.28	Y=5.24 lg(x) -1.37	Y=5.04 lg(x) +0.03
$LT_{50}$ (95% F. Limits)	3.95 (2.33~5.47)	2.09 (1.36~2.75)	1.24 (0.12~1.90)	1.83 (1.69~1.96)	0.97 (0.86~1.09)
$LT_{99}$ (95% F. Limits)	12.65 (7.98~73.27)	9.40 (5.70~41.82)	7.58 (3.74~8180.93)	5.08 (4.48~5.97)	2.86 (2.39~3.81)

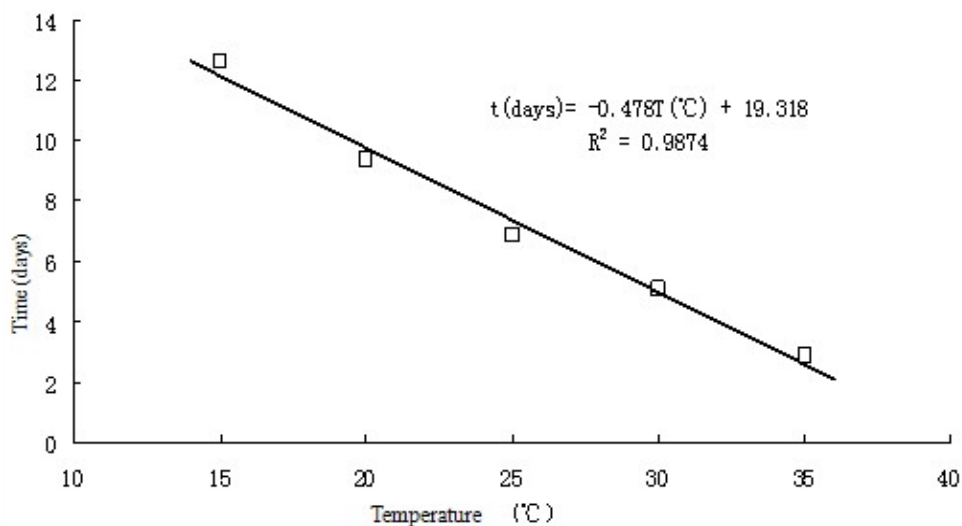


Fig. 2- The effect of temperature and exposure time to obtain LT<sub>99</sub> values for *Lasioderma serricorne* (Fabricius) exposed to 2% oxygen at 75% r.h.

### 2.2 Effect of low oxygen on *L. serricorne* larvae

At 30°C and 75% r.h. 2% oxygen 10 d exposure caused 100% mortality to *L. serricorne* larvae. Table 2 shows that a good equation fitness is obtained:  $Y=9.33 \lg(x) -6.85$ , LT<sub>50</sub> is 5.42 d, 95% Fiducial Limit is 5.18~5.66 d, LT<sub>99</sub> is 9.62 d, 95% Fiducial Limit 8.86~10.74 d, the b value of toxicity regression curve is 9.33.

Table 2. Mortality of *L. serricorne* larvae and pupae exposed to 2% oxygen at 75% r.h. and 30°C

time(d)	stages	
	larva	pupa
0	5.56± 4.01	7.78± 2.94
4	17.78±2.22	54.44±12.81
6	63.33±1.92	88.89±2.94
8	95.56±2.22	100.00±0.00
10	100.00±0.00	100.00±0.00
Reg. equation	$Y=9.33 \lg(x) -6.85$	$Y=7.71 \lg(x)-4.67$
LT <sub>50</sub> (95% F. Limits)	5.42(5.18~5.66)	4.03(3.71~4.29)
LT <sub>99</sub> (95% F. Limits)	9.62(8.86~10.74)	8.07(7.22~9.58)

### 2.3 Effect of low oxygen on *L. serricorne* pupae

At 30°C and 75% r.h. 2% oxygen 8 d exposure caused 100% mortality to *L. serricorne* pupae. Table 2 shows that a good equation fitness is obtained:  $Y=7.71 \lg(x)-4.67$ , LT<sub>50</sub> is 4.03 d.

95% Fiducial Limit is 3.71~4.29 d, LT<sub>99</sub> is 8.07 d, 95% Fiducial Limit is 7.22~9.58 d.

#### 2.4 Effect of low oxygen on *L. serricorne* adults

Table 3 shows that the biological assay equation is:  $Y=3.12 \lg(x)-1.1966$ , when the *L. serricorne* adult was treated at 35°C, 75% r.h. and 2% oxygen, the LT<sub>50</sub> is 2.42 d, 95% Fiducial Limit is 1.76-3.02 d, at LT<sub>99</sub> is 13.43 d, 95% Fiducial Limit is 8.32-40.34 d.

Tests on *L. serricorne* adults were repeated 7 times at 30°C, 75% r.h. and 2% oxygen (Table 3). However, the reproducibility was with high variation. Probit method resulting that LT<sub>99</sub> was from 5.08 d to 35 d. These results may be related to the sexual distinction and longevity difference on adults, which needs further investigation.

Results on LT values of *L. serricorne* adults at 2% oxygen, based on bioassays repeated twice under the same condition of oxygen and r.h. at 25°C are shown in Table 3. The results analyzed by Probit method could not generate biological assay equation.

In the above experiments of exposure of adults, no eggs were found in the cages, which mean there was no F1 *L. serricorne* after 2% oxygen treatment.

At the lower range of temperatures, mortality results were lower, which were not reported here.

Table 3. Mortality of *L. serricorne* adults exposed to 2% oxygen at 75% r.h. and 25-35°C

time (days)	temperature (°C.)					
	25	25	30	30	30	35
0	4.44±1.11	2.22±2.22	28.89±20.58	8.89±4.01	5.56±2.94	6.67±1.76
1	\	15.56±2.94	\	\	8.89±2.22	13.33±1.76
2	\	34.44±4.44	\	\	8.89 ±2.22	53.33±8.35
3	\	11.11±4.84	70.00±1.92	\	25.56±9.09	58.00±13.61
4	\	33.33±5.09	\	\	44.44±4.84	85.33±5.46
5	46.67±3.85	45.56±5.88	98.89 ±1.11	68.89±8.01	45.56±2.94	81.33±2.67
6	\	\	\	\	51.11±6.17	87.33±5.92
7	86.67±3.85	\	100.00±0.00	82.22±2.94	62.22±1.11	\
8	\	\	\	\	72.22±7.78	\
9	86.67±3.85	\	\	88.89±6.17	\	\
Reg. Equation	Y=5.32 lg(x) -3.76	Y=1.02 lg(x) -1.08	Y=8.23 lg(x) -3.48	Y=2.98 lg(x) -1.67	Y=2.92 lg(x) -2.19	Y=3.12 lg(x) -1.20
LT <sub>50</sub> (95% F. Limits)	5.09 (...~...)	11.30 (...~...)	2.65 (2.29~2.87)	3.64 (1.88~4.55)	5.63 (4.92~6.66)	2.42 (1.76~3.02)
LT <sub>99</sub> (95% F. Limits)	13.93 (...~...)	2115 (...~...)	5.08 (4.41~6.84)	21.93 (14.28~83.47)	35.25 (22.23~79.56)	13.43 (8.32~40.34)

#### 2.5 Comparative effects of treatment on different development stages of *L. serricorne*

Bioassay equation shown in Fig. 3 is ideally representing the four development stages of *L. serricorne* treated at 30°C, 75% r.h. and 2% oxygen.

The regression equations of LT<sub>50</sub> data of four stages are shown in Table 4 which shows that 2% oxygen has no significant difference on the LT<sub>50</sub> for pupae and adults.

Table 4. Comparison of  $LT_{50}$  values expressed by regression equations ( $\alpha=0.05$ ) on four stages of *L. serricornis*

		P			
		egg	larva	pupa	adult
$\mu$	egg	\	<0.0001	<0.0001	0.0084
	larva	25.54	\	<0.0001	0.0103
	pupa	7.72	4.58	\	0.5943
	adult	2.63	2.57	0.53	\

Whereas, differences on the sensitivity of the other two stages is remarkable (Fig. 3). Based on  $LT_{50}$  values, the sensitivity of four stages of *L. serricornis* exposed to 2% oxygen in increasing order were: eggs, adults, pupae, and larvae; and based on  $LT_{99}$  values, the sensitivity of four stages of *L. serricornis* to 2% oxygen in increasing order were: eggs, pupae, larvae, and adults.

Table 5. Comparison of slope (b) of toxicity regression equation ( $\alpha=0.05$ )

		P			
		egg	larva	pupa	Adult
$t$	egg	\	<0.0001	0.0134	0.0106
	larva	4.7642	\	0.1790	<0.0001
	pupa	2.4723	1.3439	\	0.0001
	adult	2.5569	5.7487	3.8833	\

Comparison of slopes (b) of regression equations for four stages is shown in Table 5, indicating that there were no significant differences on slopes between larvae and pupae. The pupae as a transitional stage from larvae to adults, influenced the sensitivity of adults. The sensitivity variation range inside the strain is more apt to larvae. However, the tendency of adults to influence other stages is significantly lower than that of other stages. This means that the adult species bears wider variation range of resistance to low oxygen, probably because the large difference in longevity between male and female adults. *L. serricornis* adult can live about 14~50 d (Jisheng et al, 2006), therefore, it low oxygen treatment to attain  $LT_{99}$  should be targeted in pest control strategies.

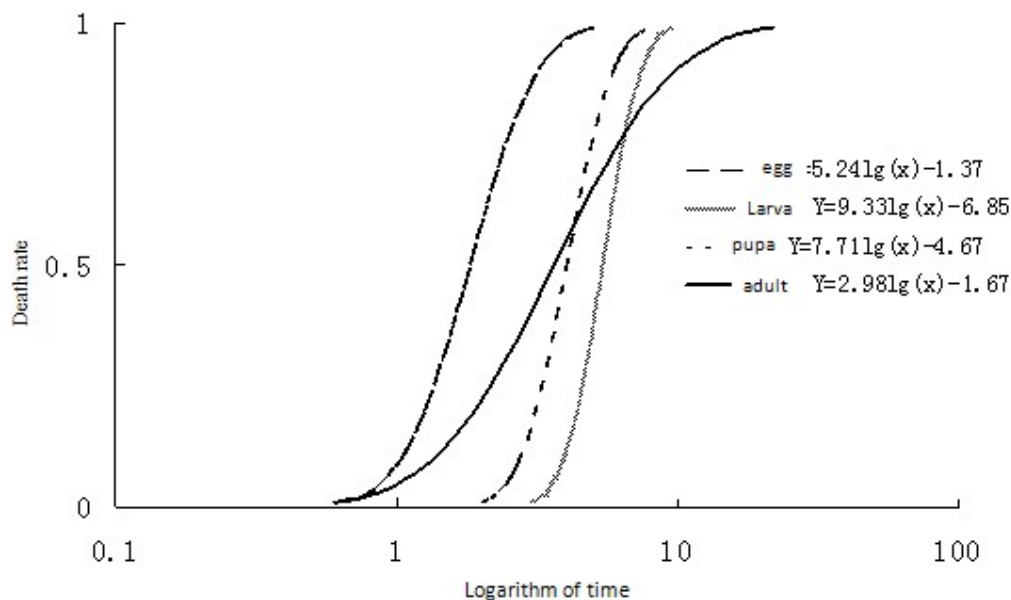


Fig. 3- Mortality of the four development stages of *Lasioderma serricorne* (Fabricius) exposed to 2% oxygen at 75% r.h. and 30°C.

### CONCLUSIONS

Low oxygen is a very effective way for control of *L. serricorne* in all four stages since 99% mortality can be achieved within 10 d of treatment of eggs, larvae, and pupae at 30°C, 75% r.h. and 2% oxygen. While for adults about 22 d of exposure same conditions produce 99% mortality. Since multiple adult tests resulted in a wide variation range of mortality, such data will need further investigation.

Each stages of *L. serricorne* bear different sensitivity to low oxygen. Based on  $LT_{50}$ , the sensitivity of four stages of *L. serricorne* to 2% oxygen in increasing order was: eggs, adults, pupae, and larvae. Based on  $LT_{99}$ , the sensitivity of four stages of *L. serricorne* to 2% oxygen in increasing order was: eggs, pupae, larvae, and adults.

Temperature plays an important role in the control of *L. serricorne* exposed to 2% oxygen. Low temperature reduces effectiveness of the treatment. For the control of the egg stage at 75% r.h., for each temperature decrease by 1°C, the treatment time increased by 0.48 d. The influence of temperature on the other stages of *L. serricorne* needs further investigation.

### REFERENCES

- Arbogast R T, Kendra P E, Mankin R W, McDonald RC (2002). Insect infestation of a botanical warehouse in north-central Florida. *Journal of Stored Products Research*, 38:349–363.
- Burton R H (1998). Sealing permanent storages for fumigation using controlled atmospheres:



- the WA experience. Stored Grain Research Laboratory (Australia), Stored Grain in Australia: Proceedings of the Australian Postharvest Technical Conference, 26-29 May 1998, Canberra:126–132.
- Conyers S T, Bell C H, Adler C S, Reichmuth Ch (1997). The effect of modified atmospheres on the juvenile stages of six grain beetles. In: Donahaye EJ, Navarro S, Vamava A (Eds.) The International Conference on Controlled Atmosphere and Fumigation, 21-26 April 1996. Printco Ltd. Nicosia Cyprus, pp: 73–81.
- Fangxiao W, Chongsheng W, Xiaoming F (1998). Study on *L. serricornis* (Fabricius). Chinese Tobacco Science ,2:45–47.
- Guangjun S, Yuzhou D, Chengyi L (2000). Directory of tobacco boring pests and natural enemies in China (A). In: L.Ryan(ed) Tobacco Storage Pest Control[C] .Earthquake Publish Company ,Beijing,pp166 - 197.
- Jianhua L, Xinhong S, Liangyue Y (2010). Study on growth and development of *L. serricornis* (Fabricius) in 9 plants materials. Journal of Henan University of Technology (Social Science Edition), 31(4):9–13.
- Jisheng M, Meihao L, Xianruo G (2006) Chinese Tobacco Pests. Science Press
- Jizhen S, Hanping Z, Jiaqin X (2006). Investigation on Loss of *L. serricornis* (Fabricius) Damage, Tobacco Science ,7: 56–58.
- Krishnamurthy T.S, Spratt E C, Bell C H (1986). The toxicity of carbon dioxide to adult beetles in low oxygen atmospheres. Journal of Stored Products Research 22:145–151.
- Ryan L (1995). Post-Harvest Tobacco Infestation Control. Kluwer Academic Publishers, Netherlands.
- Xinwen G, Guoling M, Chuanren L (1995). New study progress on *L. serricornis* (Fabricius). Journal of Hubei Agricultural College 15(3), 235–240.