J Pest Sci (2009) 82:7–11
DOI 10.1007/s10340-008-0221-1

REVIEW

Partial characterization of stress-induced carboxylesterase from adults of *Stegobium paniceum* and *Lasioderma serricorne* (Coleoptera: Anobiidae) subjected to CO_2-enriched atmosphere

Can Li · Zi Zhong Li · Yu Cao · Bo Zhou · Xingwang Zheng

Received: 11 August 2007 / Revised: 21 August 2008 / Accepted: 4 September 2008 / Published online: 2 October 2008

Abstract The kinetics effects of carbon dioxide enriched atmosphere on carboxylesterase (CarE) activity from *Stegobium paniceum* and *Lasioderma serricorne* were comparatively investigated here. The results showed that *L. serricorne* had significantly greater specific activity of CarE than *S. paniceum* [0.399 vs. 0.358 mmol (min mg)$^{-1}$]. Moreover, CarE of *L. serricorne* expressed a higher affinity (i.e. lower K_m value) to the substrate α-naphthyl acetate than *S. paniceum* (0.1 vs. 0.8 mM). The in vitro kinetics of CarE showed that there were no significant effects of controlled atmosphere on the affinity of carboxylexterase to α-naphthyl acetate for both pests besides increased of V_{max} values. Such result draws attention to the higher tolerance of *L. serricorne* to CO_2-enriched atmosphere and the necessary care required for managing this species with such a control tactic. The information also suggests a potential effect of esterases in mitigating the toxic effect of controlled atmosphere in insect pest species.

Communicated by A. Juen.

C. Li · Z. Z. Li (✉) · Y. Cao · B. Zhou · X. Zheng
Institute of Entomology, Guizhou University,
Guiyang 550025, People's Republic of China
e-mail: lizizhong38@163.com

C. Li
Department of Biology and Engineering of Environment,
Guiyang University, Guiyang 550005,
People's Republic of China
e-mail: lican790108@163.com

Keywords Carboxylesterase · Controlled atmosphere · *Lasioderma serricorne* · *Stegobium paniceum* · Susceptibility

Introduction

Chinese medicinal materials (CMM) are widely available in China. Most of these products are stored prior to use in health protection or disease treatments. However, great losses caused by insect pests occur in storage throughout the year. The drugstore beetle *Stegobium paniceum* (L.) and the cigarette beetle *Lasioderma serricorne* (Fabricius) are two closely related species of small, reddish brown beetles that are moderately common pests throughout the world. *S. paniceum* and *L. serricorne* are dominant species found in stored CMM in Hubei, Guizhou, and Shandong provinces, China (Li and Li 2007). These pest species are found in more than 300 drug materials, causing huge losses to the CMM industry in China. Measures have been taken to control their infestation (Gunasekaran and Rajendran 2005; Platt et al. 1998; Nielsen 2001; Hashem 2000). However, the effects of routine fumigations on warehouses and storage facilities with methyl bromide and phosphine are quite poor in controlling these pests. In addition, the rapid development of resistance to chemical and physical treatments by stored product pests has also been widely reported (Rajendran and Narasimhan 1994; Zettler and Keevr 1994; Wang and Zhao 2003).

For half a century, residual chemicals and fumigants have been the most effective ways of protecting and disinfesting stored products (Beckett et al. 1998). However, insect pest management in stored food commodities is facing a crisis due to increasing dissatisfaction of pesticide levels and the increasing incidences of insect resistance to

conventional chemical pesticides (Pimentel et al. 2007). These issues mean that the developments of effective alternative techniques of pest control are essential (Beckett et al. 1998). The application of controlled atmosphere (CA) (Gunasekaran and Rajendran 2005; Bell and Armitage 1992), is an effective, safe, and residue-free alternative to chemical fumigants and protectants against insects and mites (Mc Gaughey and Akins 1989; Fleurat-Lessar 1990; Annis and Morton 1997). CA treatment for stored product protection involves two main methods of producing physiological and biochemical stress in the pest organisms: an increase in the carbon dioxide (CO_2) content of the storage environment producing hypercarbia, or a reduction in the oxygen (O_2) content, obtained usually by flushing with nitrogen (N_2), producing hypoxia or anoxia (Ofuya and Reichmuth 2002). Although CA appears to have a promising future, the ability of some stored product insects to develop tolerance to CA treatments has been demonstrated in some instances (Bond and Buckland 1979; Navarro et al. 1985).

CarE efficiently catalyzes ester hydrolysis and is classified into the serine hydrolase super-family. Serine hydrolases are involved in detoxification or metabolic activation of various drugs, environmental toxicants, and carcinogens, and also play an important physiological role in lipid metabolism (Newcomb et al. 1997; Owusu et al. 1996). Metabolic resistance to organophosphorus insecticides has been associated with changes in the activity of CarE in many insect species (Devonshire and Field 1991). In two well-studied cases in which resistance to organophosphorus insecticides are associated with an increase in CarE activity, sequestration and slow turnover of the phosphate by an over-expressed esterase are responsible for resistance (Devonshire 1977; Karunaratne et al. 1993; Ketterman et al. 1993; Jayawardena et al. 1994). Extensive use of CA in insect control could lead to selection of insect populations resistant to hypercarbia and hypoxia (Donahaye 1990a, b; Wang and Zhao 2003). Several studies have demonstrated that stored product insect pests have the genetic potential to develop resistance to CA (Bond and Buckland 1979; Navarro et al. 1985; Zhao and Zhang 1993; Wang et al. 1999, 2000; Wang and Zhao 2003). Resistance to CA was related to the change of esterase activity (Wang et al. 2000) and enhanced levels of triacylglycerol and polysaccharides (Wang and Zhao 2003). As little is known about the mechanisms of CA resistance in drugstore beetle and cigarette beetle, information on the CA biochemistry of the two species' esterases will be valuable in formulating strategies in the control of these rapidly-proliferating pests. This study was initiated in order to understand the kinetics of CarE inhibition or activation by CA with (80 ± 5)% CO_2 of two pest species and it is an initial step in elucidating the molecular basis of resistance to CA.

Materials and methods

Insects

Stock colonies of drugstore beetle and cigarette beetle were established from larvae collected in a CMM warehouse in Guiyang, China in 2002. The colonies were maintained on two species of *Radix euphorbiae* Fischerianae (One piece of CMM) in a room maintained at 29 ± 1°C and a scotoperiod of 10 h. Cultures were set up in glass bottles (250 mL) with a nylon screen cover and kept in desiccators (5 L), in which the humidity was controlled with saturated NaCl solution at 75–80%. After three generations, insects from the stock colonies were used for the tests. All experiments were conducted under the conditions described above with 2–3 day-old adults of both species.

Chemicals and controlled atmosphere

α-Naphtol (Guangfu Research center, Tianjin, China), α-naphthyl acetate (α-NA), Eserine (Sigma), Fast blue B salt (Sigma), Bovine Serum Albumin (BSA, Roche), Coomassie brilliantblue G-250 (Fluka), and other biochemical reagents were of reagent grade or better. The CA with (80 ± 5)% CO_2 was used in the test.

Bioassay

The efficacy of CA against the two different beetles was determined using the small airtight glass box (10 cm × 6 cm × 4 cm). Various exposure periods of CA were tested until a satisfactory mortality range (5–99%) was ascertained. Six exposure periods were used in the final analysis.

Each bioassay consisted of 60 adults per concentration and six exposure periods (3–18 h). Control groups were exposed to natural atmospheric conditions. Mortality was assessed after pests were transferred to natural atmospheric conditions for 6 h. Pests that did not move after prodded with a camel's hair brush were scored as dead. All tests were run at 29°C and replicated at least five times on different days. Mortality data was corrected with Abbott's (1925) formula and analyzed by probit analysis (Raymond 1985) to determine the median mortality time (LT_{50}).

Enzyme preparation

For carboxylesterase (CarE), 20 adults were ground in 8 mL of ice-cold sodium phosphate buffer (0.04 M, pH 7.0) in a tissue grinder. The crude homogenates were centrifuged at 10,000*g* for 15 min at 4°C. The resulting supernatant was used as enzyme source. To assess the effect of CA on the carboxylesterase activity, 60 adults of each species

were exposed to CA for 9 h and subsequently subjected to enzyme extraction as described above.

Carboxylesterase assay

Van Asperen's (1962) method was adapted for the determination of esterase activity using α-Naphehol as standard. The general buffer was phosphate buffer (0.04 M, pH 7.0). α-naphtol (3×10^{-4} M) was used as substrate. In determining the Michaelis constants for α-naphtol, the substrate concentrations of 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 mM were made up in phosphate buffer (0.04 M, pH 7.0). The solutions were incubated at 37°C for 30 min in a water bath. The reaction was terminated by adding 1 mL of Fast Blue B salt-sodium dodecylsulphate solution. Absorbance was read in the Microplate Reader Sunrise™ after 10 min at 600 nm. The kinetic parameters (K_m and V_{max}) were determined graphically by Lineweaver-Burk plots (Wilkinson 1961).

The in vitro effect of CarE activity was ascertained using 3×10^{-4} M α-NA as substrate. The enzyme homogenate was treated with CA for 10 min in 37°C water bath. The same homogenate was treated with nature atmosphere and the same homogenate without any treatment served as a control.

Assays of protein contents

Protein contents of the enzyme homogenate were determined according to the method of Bradford (1976) using bovine serum albumin as standard. The measurement was performed with the Microplate Reader SUNRISE™ at 595 nm.

Results

Bioassays

The exposure periods of CA required to obtain LT_{50} values for *S. paniceum* and *L. serricorne* adults are summarized in Table 1. The data show that *S. paniceum* is more tolerant of CO_2 than *L. serricorne* based on LT_{50} values. However, the difference of tolerance between two species is not significant considering the 95% confidence limit.

Activities of carboxylesterase

There was a strong linear relationship between homogenate concentration and CarE activity for both *S. paniceum* ($r = 0.987$) and *L. serricorne* ($r = 0.996$). CarE from *S. paniceum* showed a significantly lower affinity (i.e. higher K_m value) to the substrate α-NA than that of *L. serricorne* ($P < 0.05$). In contrast, the catalytic activity of α-NA towards CarE in *S. paniceum* was lower (i.e. lower V_{max} value) than that in *L. serricorne*. The higher specific activity of *L. serricorne* esterase towards α-NA than that of *S. paniceum* is observed (Table 2).

Table 1 The toxicity of controlled atmosphere to *S. paniceum* and *L. serricorne*

Insects species	Slope ± SE	LT_{50} (h) (95% CL)[a]	Chi-square
S. paniceum	0.7 ± 0.1	12.4 (10.3–14.5)	2.2
L. serricorne	0.9 ± 0.2	11.4 (9.9–12.9)	2.3

[a] 95% Confident limits. LT_{50} is considered significantly different when the 95% CI fail to overlap. The tests were carried out at 29°C

The effect of CA on activities of CarE can be seen in Fig. 1. The specific activity of CarE increased with the prolonging of exposure times for both of the pest species within the sublethal period. However, the change of CarE activity from *S. paniceum* was sharper than *L. serricorne*. Such a difference could be one of the main reasons why *S. paciceum* is more tolerant to CO_2 than *L. serricorne*. The results of in vitro CarE assays by CA were shown at Table 3. The affinities of CarE towards α-NA were not significantly affected in either *S. paniceum* or *L. serricorne*, while the catalytic activity of CarE towards α-NA was improved when treated with CO_2-enriched atmosphere for both pests.

Discussion

According to Gunasekaran and Rajendran (2005), all life stages of *S. paniceum* were relatively more susceptible than those of *L. serricorne*. However, the current research showed that the adults of *S. paniceum* are more tolerant of CA than *L. serricorne* based on LT_{50} values, although the difference of tolerance between the two species is not significant considering the 95% confidence limit (Table 1). The greater specific activity of *L. serricorne* esterase towards α-NA indicated that CarE extracted from *L. serricorne* has a stronger detoxifying capability to exogenous

Table 2 Carboxylesterase activity and kinetic parameters using α-NA as substrates in *S. paniceum* and *L. serricorne*

Insects species	Specific activity [mmol (min mg)$^{-1}$]	V_{max} [mmol (mg min)$^{-1}$]	K_m (mmol/L)
S. paniceum	0.358[b]	1.2 ± 0.1[b]	0.8 ± 0.07[a]
L. serricorne	0.398[a]	3.1 ± 0.5[a]	0.3 ± 0.08[b]
Ratio	1.1	2.5	2.7

Means followed by the same letter within a column are not significantly different by Paired Samples Test at $P < 0.05$. The tests were carried out at 29°C

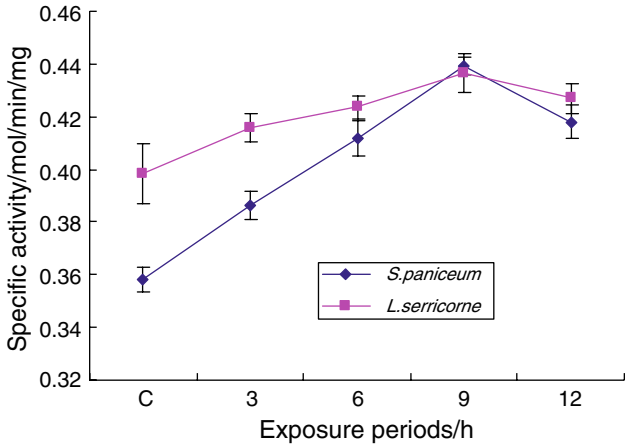

Fig. 1 Relationship between CarE activity and exposure time to CA. Sixty adults of each species were exposed to CA for 3, 6, 9 and 12 h. The subsequent carboxylesterase assays were repeated 3 times. The control group (*C*) was exposed to natural atmospheric conditions. Tests were carried out at 29°C

Table 3 The in vitro effects of controlled atmosphere on kinetic parameters of CarE using α-NA as substrates in *S. paniceum* and *L. serricorne*

Insects species	Treated	V_{max} [mmol (mg min)$^{-1}$]	K_m(mmol/L)
S. paniceum	CO_2	2.705 ± 0.028^a	0.037 ± 0.011^a
	CK	2.025 ± 0.019^b	0.061 ± 0.012^a
	Ratio	1.33	1.64
L. serricorne	CO_2	1.845 ± 0.016^a	0.066 ± 0.011^a
	CK	1.130 ± 0.034^b	0.095 ± 0.024^a
	Ratio	1.63	1.44

Means followed by the same letter within a column for the same species are not significantly different by paired samples test at $P < 0.05$. CO_2 means the enzyme homogenate was treated with CA for 10 min in 37°C water bath. CK Means the same homogenate was treated under natural atmospheric conditions

chemicals than that of *S. paniceum*. Esterases are detoxification enzymes, which may lead to insecticide resistance to organophosphate, carbamate and pyrethroid insecticides in different insects or mites. Increased CarE activity in insects resistant to organophosphorus insecticides have been well documented (Liu et al. 2005; Hama and Hosoda 1983; Wang et al. 1999, 2000). For both species, *S. paniceum* and *L. serricorne*, there have been no reports about the relationship between CarE and CA. Based on biological research, the catalytic activity of CarE towards α-NA were improved after treatment with CO_2-enriched atmosphere for both species. Pest resistance to CA was related to the change of activity of esterase (Wang et al. 2000) and enhanced levels of triacylglycerol and polysaccharides (Wang and Zhao 2003). The lethal efficiency of CA to the pests may be related to activity change of CarE. The current work showed that improved CarE activity in *S. panuceum* and *L. serricorne* may result from extended exposure to CA. These results are different from those with conventional insecticides, which usually lead to a decrease in CarE activity in susceptible insects, but enhanced activity in insecticide-resistant individuals (Wang et al. 2004). The results of the kinetics revealed that CarE in *S. paniceum* showed a significantly lower affinity to the substrate α-NA than that of *L. serricorne*. In contrast, the catalytic activity of CarE towards α-NA in *S. paniceum* was lower than that in *L. serricorne*. These differences may be contributing to the difference of detoxification or build up of insecticide resistance. In vitro kinetic result from CO_2-atmosphere suggested that there is a relationship between CA and V_{max} of CarE from the two insects; it was indicated that CarE could partly hydrolyze toxic products produced by CA in *S. paniceum* and *L. serricorne*. The CarE induction by CA was higher in *L. serricorne* suggesting a greater potential of this species to develop resistance to CA.

The present study provided some basic information on carboxylesterases and their induction by CA in two species of Anobiidae beetles, which are likely to be useful to understand the mechanisms underlying the tolerance to CO_2-enriched atmospheres. As information about other Anobiidae species, as well as other stored product beetles, exhibiting some tolerance to CA becomes available it will be valuable to carry out further toxicological and biochemical studies.

Acknowledgments The authors would like to thank Professor Wang JJ, Southwest University, China, for checking the manuscript. This research was funded in part by "the tenth" key program of agricultural science and technology [No. 20011110], Natural Science Foundation of Educational Office [No. 2007061], Guiyang city science and technology bureau [No. 2007 (6–4)], Guizhou Province, China.

References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265–267

Annis PC, Morton R (1997) The acute mortality effects of carbon dioxide on various life stages of *Sitophilus oryzae*. J Stored Prod Res 33:115–124. doi:10.1016/S0022-474X(96)00050-1

Beckett SJ, Morton R, Darby JA (1998) The mortality of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) at moderate temperatures. J Stored Prod Res 34:363–376. doi:10.1016/S0022-474X(98)00022-8

Bell CH, Armitage DM (1992) Alternative storage practices. In: Saur DB (ed) Storage of cereal grains and their products, 4th edn edn. American Association of Cereal Chemists, Minnesota, pp 249–311

Bond EJ, Buckland CT (1979) Development of resistance of carbon dioxide in the granary weevil. J Econ Entomol 60:878–879

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of

protein-dry binding. Anal Biochem 72:248–254. doi:10.1016/0003-2697(76)90527-3

Devonshire AL (1977) The properties of a carboxylesterase from the peach-potato aphid, *Myzus persicae* (Sulz.), and its role in conferring insecticide resistance. Biochem J 167:675–683

Devonshire AL, Field LM (1991) Gene amplification and insecticide resistance. Annu Rev Entomol 36:1–21. doi:10.1146/annurev.en.36.010191.000245

Donahaye E (1990a) Laboratory selection of resistance by the red flour beetle *Tribolium castaneum* (Herbst) to a carbon dioxide-enriched atmosphere. Phytoparasitica 18:229–308

Donahaye E (1990b) The potential for stored-product insects to develop resistance to modified atmosphere. In: Fleurat-Lessard F, Ducom, Proceeding of fifth international working conference on stored product protection, 9–14 September 1990. Bordeaux, France, pp 989–996

Fleurat-Lessard F (1990) Effects of modified atmosphere on insects and mites infesting stored products. In: Calderon M, Burkai-Golan R (eds) Food preservation by modified atmospheres. CRC Press, Boca Raton, pp 21–38

Gunasekaran N, Rajendran S (2005) Toxicity of carbon dioxide to drugstore beetle *Stegobium paniceum* and cigarette beetle *Lasioderma serricorne*. J Stored Prod Res 41:283–294. doi:10.1016/j.jspr.2004.04.001

Hama H, Hosoda A (1983) High aliesterase activity and low acetylcholinestease sensitivity involved in organophosphorus and carbamate resistance of the brown planthopper, *Nilaparvata lugens* Stål (Homoptera: Delphacidae). Appl Entomol Zool (Jpn) 18:475–485

Hashem MY (2000) Suggested procedures for applying carbon dioxide (CO_2) to control stored medicinal plant products from insect pests. Z Pflanzenk Pflanzen 107:212–217

Jayawardena KGI, Karunaratne SHPP, Ketterman AJ, Hemingway J (1994) Determination of the role of elevated B_2 esterase in insecticide resistance in *Culex quinquefasciatus* (Diptera: Culicidae) from studies on the purified enzyme. Bull Entomol Res 84:39–44

Karunaratne SHPP, Jayawardena KGI, Hemingway J, Ketterman AJ (1993) Characterisation of a B-type esterase involved in insecticide resistance from the mosquito *Culex quinquefasciatus*. Biochem J 294:575–579

Ketterman AJ, Karunararne SHPP, Jayawardena KGI (1993) Qualitative differences between populations of *Culex quinquefasciatus* in both the esterases A_2 and B_2 which are involved in insecticide resistance. Pestic Biochem Physiol 47:142–148. doi:10.1006/pest.1993.1072

Li C, Li ZZ (2007) The development threshold temperature and the effective accumulated temperature of *Stegobium paniceum*. Chin Bull Entomol 44:379–381

Liu X, He YP, Ma EB (2005) Characterization and comparison of general esterases from two field populations of the oriental migratory locust, *Locusta migratoria manilensis* (Meyen) (Orthoptera: Acrididae) from Shanxi Province. Acta Entomol Sin 48:24–30

Mc Gaughey WH, Akins RG (1989) Application of modified atmospheres in farm grain storage bins. J Stored Prod Res 25:201–210. doi:10.1016/0022-474X(89)90025-8

Navarro S, Dias R, Donahaye E (1985) Induced tolerance of *Sitophilus oryzae* adults to carbon dioxide. J Stored Prod Res 24:207–213. doi:10.1016/0022-474X(85)90017-7

Newcomb RD, Campbell PM, Russell RJ, Oakeshott JG (1997) cDNA cloning, baculovirus-expression and kinetic properties of the esterase, E3, involved in organophosphorus resistance in *Lucilia cuprina*. Insect Biochem Mol Biol 27:15–25. doi:10.1016/S0965-1748(96)00065-3

Nielsen PS (2001) The effect of carbon dioxide under pressure against eggs of *Ephestia kuehniella* Zeller and the adults of *Stegobium paniceum* (L.) and *Oryzaephilus surinamensis* (L.). Anz Schadl J Pest Sci 74:85–88

Ofuya TI, Reichmuth C (2002) Effect of humidity on the susceptibility of Callosobruchus maculates (Fabricius) (Coleoptera: Bruchidae) to two modified atmospheres. J Stored Prod Res 38:139–146. doi:10.1016/S0022-474X(01)00009-1

Owusu EO, Horiike M, Hirano C (1996) Polyacrylamide gel electrophoretic assessments of esterase in cotton aphid (Homoptera: Aphididae) resistance to dichlorvos. J Econ Entomol 89:302–306

Pimentel MAG, Faroni LRD`A, Tótola MR, Guedes RNC (2007) Phosphine resistance, respiration rate and fitness consequences in stored-product insects. Pest Manag Sci 63:876–881. doi:10.1002/ps.1416

Platt RR, Cuperus GW, Payton ME, Bonjour EL, Pinkston KN (1998) Integrated pest management perceptions and practices and insect populations in grocery stores in South-central United States. J Stored Prod Res 34:1–10. doi:10.1016/S0022-474X(97)00036-2

Rajendran S, Narasimhan KS (1994) Phosphine resistance in the cigarette beetle *Lasioderma serricorne* (Coleoptera Anobiidae) and overcoming control failures during fumigation of stored tobacco. Int J Pest Manage 40:207–210

Raymond M (1985) Présentation d'un programme Basic d'analyse log-probit pour micro-ordinateur. Cahiers ORSTOM, série Entomologie médicale et Parasitologie 23:117–121

Van Asperen K (1962) A study of house fly esterase by means of a sensitive colorimetric method. J Insect Physiol 8:401–416. doi:10.1016/0022-1910(62)90074-4

Wang JJ, Zhao ZM (2003) Accumulation and utilization of triacylglycerol and polysaccharides in *Liposcelis bostrychophila* (Psocoptera, Liposcelididae) selected for resistance to carbon dioxide. J Appl Ent 127:107–111. doi:10.1046/j.1439-0418.2003.00718.x

Wang JJ, Zhao ZM, Li LS (1999) Induced tolerances of the psocid, *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae), to controlled atmosphere. Int J Pest Manage 45:75–79. doi:10.1080/096708799228085

Wang JJ, Zhao ZM, Tsai JH (2000) Resistance and some enzyme activities in *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae), in relation to carbon dioxide enriched atmosphere. J Stored Prod Res 36:297–308. doi:10.1016/S0022-474X(99)00051-X

Wang JJ, Cheng WX, Ding D, Zhao ZM (2004) Effect of the insecticide dichlorvos on esterase activity extracted from the psocide, *Liposcelis bostrychophila* and *L. entomophila*. J Insect Sci 4:23

Wilkinson GN (1961) Statistical estimation in enzyme kinetics. Biochem J 803:324–332

Zettler JL, Keevr DW (1994) Phosphine resistance in the cigarette beetle (Coleoptera: Anobiidae) associated with tobacco storage in the southeastern United States. J Econ Entomol 87:546–550

Zhao ZM, Zhang XW (1993) Development of resistance of *Tyrophagus putrescentiae* to controlled atmosphere. Acta Arachnol Sin 2:126–128