Is low-oxygen CA applicable to phosphine-resistant cigarette beetles?

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Abstract

Low-oxygen (O_2) controlled atmosphere (CA) technology has recently been employed in the tobacco industry as a disinfestation alternative to phosphine fumigation as resistance emerges in pests. The present CA protocol for stored tobacco was established based on experimental data obtained using a phosphine-susceptible culture of *Lasioderma serricorne*, in which the effect on phosphine-resistant populations had not been examined sufficiently. In this study, the susceptibilities of 15 laboratory cultures to phosphine and to low O_2 were examined to check the effectiveness of the CA protocol against *L. serricorne*, irrespective of phosphine resistance. The LC_{50} values of phosphine exhibited a broad range (6.6–845.3 ppm in eggs, 72-h exposure at 25°C), whereas the LT_{50} of 0.5% O_2 had a narrow range (5.4–7.6 d; eggs at 20°C). There was no correlation between the susceptibilities to low O_2 and to phosphine (r = 0.06, P = 0.982). The reference culture used to establish the protocol was the least tolerant of phosphine and the most tolerant of low O_2. These results strongly suggest that cross-resistance has not evolved and that the present low-O_2 CA protocol is useful for disinfestation of *L. serricorne*, irrespective of phosphine resistance.

Keywords: *Lasioderma serricorne*, hypoxia, controlled atmosphere, phosphine, cross-resistance

1. Introduction

Phosphine fumigation has been widely used to eradicate insect pests of stored tobacco since the mid-1970s (Ryan, 1999). However, the emergence of resistance in the cigarette beetle, *Lasioderma serricorne* (F.), the most important pest, as well as growing public concern about toxic chemicals in the environment has necessitated that the tobacco industry develop alternative technologies (Rajendran and Narasimhan 1994; Zettler and Keever 1994). Low-oxygen (O_2) controlled atmospheres (CAs) have been the subject of intense interest as an ecologically friendly alternative to phosphine fumigation. The recommended protocol for low-O_2 CAs to disinfest stored tobacco (0.5% O_2; 9-d exposure at 28°C or 4-d exposure at 38°C) was approved recently by the tobacco industry (CORESTA, 2013a). However, the protocol was developed based on experimental data obtained using phosphine-susceptible insects; the efficacy of low-O_2 CAs against phosphine-resistant populations has not been sufficiently tested. To check the validity of the protocol for *L. serricorne* irrespective of phosphine resistance, we examined the susceptibility of 15 laboratory cultures to phosphine and to low O_2 (hypoxia), respectively, and the potential for cross-resistance by using measures of correlation.

2. Materials and Methods

2.1. Insects

Fifteen laboratory cultures of *L. serricorne* were used in this study (R, C1–14). These cultures were originally collected in tobacco warehouses at seven different locations and dates. They had been maintained on 10% yeast-added cornmeal at 27°C, 60% r.h., and a photoperiod of 12:12 (L:D). More than 200 adults per culture that emerged on the rearing media were collected and enclosed individually in polystyrene cases (72 × 129 mm ϕ). A spoonful of cocoa powder (*ca*. 1 g) was put into each case. The cases were kept at 27°C and 60% r.h. for 20 h for oviposition. Eggs deposited in cocoa were sieved with a 150-µm mesh screen and used for tests.

2.2. Phosphine-susceptibility testing

The phosphine susceptibility of eggs was evaluated as described by Hori and Kasaishi (2005) except for egg preparation. One hundred eggs (collected within 24 h of being laid) were placed on the sticky face of adhesive paper mounted in a plastic Petri dish (13 × 40 mm ID). The Petri dish was then placed into a polyvinyl chloride chamber (160 × 295 × 100 mm) connected to a fumigation test apparatus (Kanto Kogyo Co., Ltd., Kanagawa, Japan) (Fig. 1). Phosphine was generated from aluminum phosphide (Tyvek®; Degesch Japan Co. Ltd., Saitama, Japan) and introduced into the test chamber after adjusting concentrations (5, 10, 50, 100, 200, 300, 500, 700, and 1000 ppm) using a phosphine gas analyzer (Komyo Rikagaku Kogyo K.K., Kanagawa, Japan). A cup of a saturated solution of sodium chloride was placed in the test chamber to maintain the relative humidity at 75%. The eggs were exposed to phosphine for 72 h at 25°C. After exposure, the eggs were maintained at 27°C, 60% r.h., and L:D 12:12 for two weeks. Egg viability was assessed by their hatching.

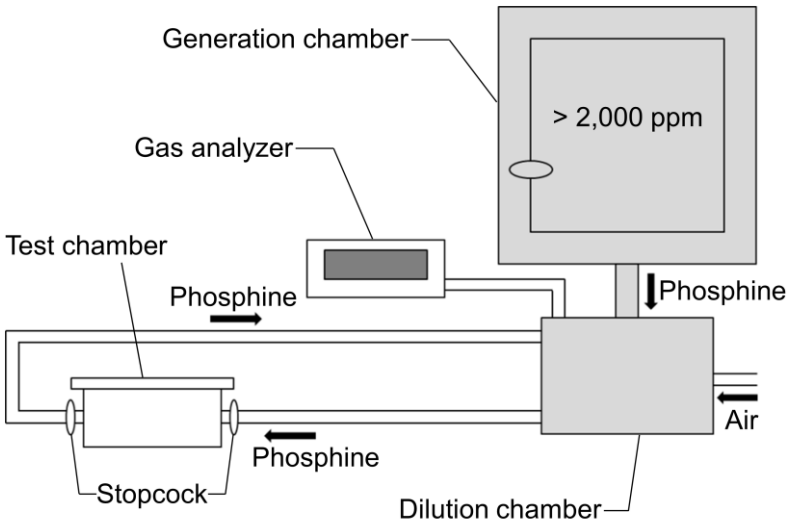

Figure 1 Fumigation test apparatus. Phosphine (> 2,000 ppm) is generated from aluminum phosphide in a generation chamber and adjusted to prescribed concentrations (5, 10, 50, 100, 200, 300, 500, 700, and 1000 ppm) in a dilution chamber using a gas analyzer. The concentration-adjusted gas is introduced into a test chamber containing the insects. After the gas introduction, the test chamber is detached from the system and placed into an incubator kept at 25°C.

2.3. Hypoxia-susceptibility testing

The hypoxia testing methods were almost identical to those described previously (Imai and Fukazawa, 2012). One hundred eggs (collected within 24 h of being laid) in glass tubes (50 × 6 mm ID) were placed into gas-washing bottles (1 L) for exposure to hypoxia. Six bottles containing eggs were serially connected by silicone tubing and purged with premixed gases (0.5% O_2 in nitrogen (N_2); Saisan Co., Ltd., Saitama, Japan) at a rate of 0.2 L/min. Humidity was controlled to 75% r.h. by passing the gas through a gas-washing bottle containing saturated solutions of sodium chloride. Tests were conducted in a temperature-controlled room (20°C). The temperature, humidity, and oxygen concentration were measured at the distal end (i.e., the longest distance from the gas source) of the series of bottles using a LogStick LS300-TH thermohygrorecorder (Osaka Micro Computer Inc., Osaka, Japan) and a GP-200 industrial oximeter (Iijima Electronics Corp., Aichi, Japan). The bottles were disconnected sequentially from the distal end at predetermined intervals (4, 6, 8, 10, and 12 d of exposure). Egg viability was checked as described in section 2.2.

2.4. Data analysis

The phosphine concentration necessary to achieve 50% lethality (LC_{50}) or the time necessary to achieve 50% lethality (LT_{50}) was calculated using the PriProbit (ver. 1.63) computer program developed by Sakuma (1998), which was downloaded from http://www.ars.usda.gov/News/docs.htm?docid=11284. Pearson's correlation coefficient (*r*) was calculated using software (PASW Statistic 18; SPSS Inc., Chicago, IL).

3. Results and Discussion

3.1. Phosphine susceptibility

The lethal concentrations of phosphine differed widely among cultures: 6.6–845.3 ppm in LC_{50} (1–128.1 in the resistance ratio) (Table 1). Phosphine resistance in *L. serricorne* was first recorded in India and the United States in 1990s (Rajendran and Narasimhan 1994; Zettler and Keever 1994), and spread worldwide by accompanying international tobacco distribution. The development of resistance to phosphine has been documented in several major cosmopolitan coleopteran pests of stored products (Bell, 2014). The resistance levels in *L. serricorne* revealed in this study were comparable to those observed in Brazilian populations of four pests, *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.), *Oryzaephilus surinamensis* (L.), and *Sitophilus zeamais* Motschulsky, with respective resistance ratios as high as 186-fold, 71-fold, 32-fold, and 87-fold (Pimentel et al., 2007; 2009). However, they were much lower than that of Australian populations of *Cryptolestes ferrugineus* (Stephens), which shows resistance ratios > 1,000-fold (Nayak et al., 2012). In the tobacco industry, resistance of *L. serricorne* has been successfully managed by increasing the exposure time and concentration (CORESTA, 2013b), but the future prospects of phosphine fumigation are not clear.

Table 1 Phosphine concentrations necessary to achieve 50% lethality (LC_{50}) and resistance ratios of eggs of *Lasioderma serricorne* cultures exposed for 72 h at 25°C and 75% r.h.

Culture	Slope ± SE	LC_{50} (95% confidence interval), ppm	Resistance ratio[a]
R	2.42 ± 0.67	6.6 (3.0–10.3)	1.0
C1	1.24 ± 0.09	7.2 (5.3–9.3)	1.1
C2	1.21 ± 0.13	7.5 (4.2–11.6)	1.1
C3	1.77 ± 0.30	136.6 (81.1–192.7)	20.7
C4	2.89 ± 0.57	147.9 (91.7–199.2)	22.4
C5	3.54 ± 0.55	266.2 (212.3–317.6)	40.4
C6	4.16 ± 0.78	463.5 (361.6–570.7)	70.3
C7	2.94 ± 0.36	717.3 (631.0–833.7)	108.7
C8	4.24 ± 0.39	250.4 (227.2–272.6)	38.0
C9	7.02 ± 1.01	340.2 (305.7–375.1)	51.6
C10	6.23 ± 1.00	284.5 (248.3–318.9)	43.1
C11	5.78 ± 0.79	298.1 (264.9–331.0)	45.2
C12	5.79 ± 0.77	391.6 (348.3–435.8)	59.4
C13	5.92 ± 1.03	412.6 (333.1–474.1)	62.5
C14	5.72 ± 0.78	845.3 (789.5–914.4)	128.1

[a]Resistance ratios were calculated using respective LC_{50} values in relation to the reference culture (R).

3.2. Hypoxia susceptibility

The reference culture (R) used to establish the CA protocol for tobacco was the most tolerant of low oxygen. Smaller differences in hypoxia susceptibility were found among cultures, with LT_{50} values ranging from 5.1 d to 7.6 d in (0.7–1.0-fold in the resistance ratio) (Table 2). The results suggest that little inherent heterogeneity in susceptibility to hypoxia exists in *L. serricorne* populations. Although the hypoxia-resistant cultures were obtained experimentally through laboratory selection in *T. castaneum* (Donahaye, 1992) and *Drosophila melanogaster* Meigen (Zhou et al., 2007), low-O_2 CAs can be used at present to control *L. serricorne* without consideration of resistance problems.

Table 2 Exposure times necessary to achieve 50% lethality (LT_{50}) and resistance ratios of eggs of *Lasioderma serricorne* cultures exposed to 0.5% O_2 at 20°C and 75% r.h.

Culture	Slope ± SE	LT_{50} (95% confidence interval), d	Resistance ratio[a]
R	2.42 ± 0.67	7.6 (6.9–8.3)	1.0
C1	1.24 ± 0.09	6.4 (5.8–7.0)	0.8
C2	1.21 ± 0.13	6.6 (6.0–7.3)	0.9
C3	1.77 ± 0.30	5.8 (5.2–6.3)	0.8
C4	2.89 ± 0.57	6.8 (6.2–7.4)	0.9
C5	3.54 ± 0.55	5.4 (4.9–5.9)	0.7
C6	4.16 ± 0.78	7.3 (6.6–8.0)	1.0
C7	2.94 ± 0.36	7.6 (7.0–8.4)	1.0
C8	4.24 ± 0.39	6.6 (6.0–7.2)	0.9
C9	7.02 ± 1.01	5.9 (5.3–6.5)	0.8
C10	6.23 ± 1.00	5.7 (5.2–6.3)	0.7
C11	5.78 ± 0.79	5.4 (4.9–6.0)	0.7
C12	5.79 ± 0.77	5.1 (4.6–5.6)	0.7
C13	5.92 ± 1.03	6.1 (5.5–6.7)	0.8
C14	5.72 ± 0.78	6.1 (5.6–6.7)	0.8

[a]Resistance ratios were calculated using respective LT_{50} values in relation to the reference culture (R).

3.3. Relation between phosphine-susceptibility and hypoxia-susceptibility

No significant correlation was found between the susceptibilities to phosphine and to hypoxia (r = 0.06, P = 0.982) (Fig. 2). An absence of cross-resistance between phosphine and hypoxia was also reported for *T. castaneum* (Donahaye, 1990) and *R. dominica* (Hasan et al., 2004). The R culture was the least tolerant of phosphine and the most tolerant of hypoxia. These results strongly suggest that cross-resistance has not evolved and that the present low-O_2 CA protocol is useful for disinfestation of *L. serricorne*, irrespective of phosphine resistance.

Physiological studies of phosphine resistance in four coleopteran stored product pests, *T. castaneum*, *R. dominica*, *O. surinamensis*, and *S. zeamais*, have revealed a significant inverse correlation between phosphine resistance and respiration rate (Pimentel et al., 2007; 2009). These findings suggest that phosphine-resistant pests require less oxygen and that they might tolerate lower-O_2 atmospheres. However, our present results suggest that the reduced oxygen demand in phosphine-resistant pests will not allow them to tolerate extreme low-oxygen environments.

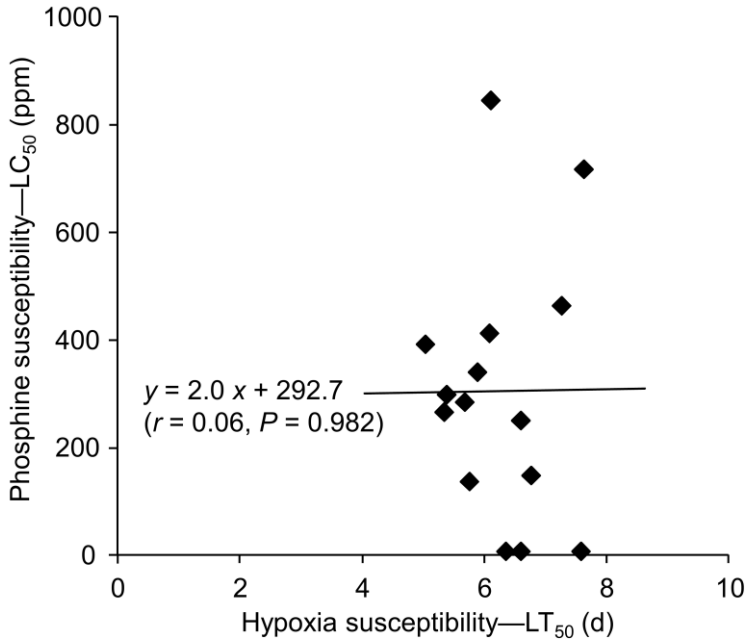

Figure 2 Relationship between phosphine susceptibility (LC_{50} at 25°C/72 h) and hypoxia susceptibility (LT_{50} at 0.5% O_2/20°C) of *Lasioderma serricorne* cultures.

References

Bell, C.H., 2014. A review of insect responses to variations encountered in the managed storage environment. Journal of Stored Products Research (In press), DOI: 10.1016/j.jspr.2014.06.004

CORESTA, 2013a. Controlled atmosphere parameters for the control of cigarette beetle and tobacco moth (Revised May 2013). http://www.coresta.org/Guides/Guide-No12_Controlled-Atmosphere_May13.pdf (Accessed November 21, 2014)

CORESTA, 2013b. Phosphine fumigation parameters for the control of cigarette beetle and tobacco moth (Revised October 2013). http://www.coresta.org/Guides/Guide-No02-Fumigation_Oct13.pdf (Accessed November 21, 2014)

Donahaye, E., 1990. The potential for stored-product insects to develop resistance to modified atmospheres. In: Fleurat-Lessard, F.; Ducom, P. (Eds.), Proceedings of the 5th International Working Conference on Stored-Product Protection, 9-14 September 1990, Bordeaux, France. Imprimerie du Médoc, Bordeaux, France, 1991, pp. 989-997.

Donahaye, E., 1992. Laboratory selection of resistance by the red flour beetle, *Tribolium castaneum* (Herbst), to an atmosphere of low oxygen concentration. Phytoparasitica 18, 189-202.

Hasan, M.M., Reichmuth, C.H., Adler, C., 2004. Efficacy of controlled atmospheres against phosphine resistant strains of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) originating from different countries. In: Donahaye, E.J., Navarro, S., Bell, C., Jayas, D., Noyes, R., Phillips, T.W. (Eds.), Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products, Gold-Coast Australia. 8-13 August 2004. FTIC Ltd. Publishing, Israel, 2007, pp. 55-67.

Hori, M., Kasaishi, Y., 2005. Development of a new assay method for quickly evaluating phosphine resistance of the cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), based on knockdown of the adult beetles. Applied Entomology and Zoology 40, 99-104.

Imai, T., Fukazawa, N., 2012. Susceptibility of the cigarette beetle *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) to hypoxia. Applied Entomology and Zoology 47, 429-432.

Nayak, M.K., Holloway, J.C., Emery, R.N., Pavic, H., Bartlet, J., Collins, P.J., 2013. Strong resistance to phosphine in the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae): its characterisation, a rapid assay for diagnosis and its distribution in Australia. Pest Management Science 69, 48-53.

Pimentel, M.A.G., Faroni, L.R.D'A., Tótola, M.R., Guedes, R.N.C., 2007. Phosphine resistance, respiration rate and fitness consequences in stored-product insects. Pest Management Science 63, 876-881.

Pimentel, M.A.G., Faroni, L.R.D'A., Guedes, R.N.C., Sousa, A.H., Tótola, M.R., 2009. Phosphine resistance in Brazilian populations of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Journal of Stored Product Research 45, 71-74.

Rajendran, S., Narasimhan, K.S., 1994. Phosphine resistance in the cigarette beetle *Lasioderma serricorne* (Coleoptera: Anobiidae) and overcoming control failures during fumigation of stored tobacco. International Journal of Pest Management 40, 207-210.

Ryan, L. (Ed.), 1999. Post-harvest tobacco infestation control. Kluwer Academic Publishers, Dordrecht.

Sakuma, M., 1998. Probit analysis of preference data. Applied Entomology and Zoology 33, 339-347.

Zettler, J.L., Keever, D.W., 1994. Phosphine resistance in cigarette beetle (Coleoptera: Anobiidae) associated with tobacco storage in the southeastern United States. Journal of Economic Entomology 87, 546-550.

Zhou, D., Xue, J., Chen, J., Morcillo, P., Lambert, J.D., White, K.P., Haddad, G.G., 2007. Experimental selection for *Drosophila* survival in extremely low O_2 environment. PLoS One 2(5), e490.