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Capability and limitation of anoxic treatments in museum collections protection

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Without precaution, insects may cause serious damage to museum collections. Quarantine of potentially infested objects can be logistically challenging. Anoxia under controlled nitrogen atmosphere is a most compatible but also time-consuming method to eradicate insect pests in all kinds of different materials. Treatment results are usually effected by duration, temperature, humidity and residual oxygen content. During a two-year research project, 34 relevant pest insect species of all developmental stages were tested in several different materials (wood, paper, wool) to monitor treatment success and to determine optimum treatment parameters. Duration of treatment ranged from one to three weeks at temperatures of 20 - 27 °C. As expected, results showed significant differences in mortality among tested species. Highest tolerance of hypoxic conditions was found in older larvae of *Hylotrupes bajulus*. However, this species is an unlikely museum pest. Anobiids and other wood boring beetles are more often an issue related to cultural heritage. Tested imbedding materials in general had no mortality lowering influence. A combination of three weeks exposure time at up to 0.5 % residual oxygen and at 24 °C and 50 % RH is recommended for infested artefacts.

Susceptibility of phosphine-resistant cigarette beetles to various insecticides

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Abstract

Management of phosphine resistance in the cigarette beetle *Lasioderma serricorne* (F.) has become a topic of great interest to the tobacco industry in recent years. Effective use of contact insecticides with modes of action different from that of phosphine can be a key element in preventing or delaying the evolution of phosphine resistance. This study was conducted to ascertain whether five insecticides selected from three mode-of-action classes (fenitrothion, pirimiphos-methyl, permethrin, bifenthrin, and spinosad) can be incorporated into a phosphine-resistance management strategy. Specifically, we examined the contact efficacy of the insecticides to a phosphine-susceptible strain and six resistant strains (38–184-fold in resistance ratio based on LC_{50}). Susceptibility to organophosphates (fenitrothion, pirimiphos-methyl) and spinosad was not significantly different between phosphine-susceptible and phosphine-resistant strains (within 2.3-fold resistance ratio). The absence of the cross-resistance between these insecticides and phosphine makes them ideal for resistance-management programmes. However, high resistance to synthetic pyrethroids (>145-fold for permethrin and >1697-fold for bifenthrin) was found in three of six phosphine-resistant strains. Based on these results, synthetic pyrethroids cannot be recommended as insecticides of primary choice.

Keywords: *Lasioderma serricorne*, resistance management, contact insecticides, pyrethroid resistance

1. Introduction

The cigarette beetle, *Lasioderma serricorne* (F.) is the most important pest of stored tobacco. Fumigation by phosphine, the most important method for disinfestation of stored tobacco, has been used for post-harvest management of insect pests since the 1970s. Phosphine resistance in *L. serricorne*, although first recorded in India and the United States in the 1990s (ZETTLER, 1990;

Rajendran and Narasimhan, 1994; Zettler and Keever, 1994), has spread globally along with international tobacco distribution (Hori and Kasaishi, 2005; CORESTA, 2013). The tobacco industry had successfully managed the resistance problem by revising the industrial fumigation protocol (CORESTA, 2013). Nevertheless, highly resistant populations able to survive the present fumigation protocol, i.e., 6-d exposure with 600-ppm at 25°C, have emerged recently in the United States (Sağlam et al., 2015). The sustainable use of phosphine will be threatened if such high resistance becomes widespread. Therefore, countermeasures capable of preventing or delaying the elevation of resistance are eagerly sought. The emergence and spread of resistance are mainly attributed to heavy reliance on a single insecticide or single mode-of-action insecticides. Therefore, enforcement of an integrated approach incorporating as many different control measures as possible is a principle for successful management of phosphine resistance. The use of insecticides with different modes of action is one such measure. Various contact insecticides, including organophosphates and pyrethroids, have been used as space-spray or surface-spray applications in tobacco warehouses in practice (Ryan, 1999), although their efficacy against phosphine-resistant *L. serricorne* has not been ascertained. The present study assesses the effects of five contact insecticides selected from three mode-of-action classes (fenitrothion, pirimiphos-methyl, permethrin, bifenthrin, and spinosad) (IRAC, 2017), to phosphine-susceptible and phosphine-resistant *L. serricorne*.

2. Materials and Methods

a. Insects

Seven laboratory strains of *L. serricorne*, one phosphine-susceptible and six phosphine-resistant strains, were used for this study (Table 1). The phosphine-susceptible strain TSC has been maintained in the laboratory for decades without exposure to any insecticide. The other six strains (THR, C87, NGY, IWT, SKG, and MLY) were established from phosphine-reistant field populations collected from tobacco warehouses at different times and locations. They underwent laboratory selection with phosphine for at least 13 generations before the study so that uniformity of phosphine susceptibility in individuals in each strain was promoted. They had never exposed to insecticides, except for phosphine, since their collection from the field. All insects were maintained on cornmeal containing yeast (10% mass fraction) at 27°C and 60% r.h.

Table 1 *Lasioderma serricorne* strains used for the study

Strain	Initiation of culture	Origin	Phosphine-susceptibility, LC_{50}[a] (95% confidence interval), ppm	
TSC	Unknown	Unknown	6.6	(3.0–10.3)
THR	1999	Tobacco warehouse in Tokyo, Japan	250.4	(227.2–272.6)
C87	2011	Food and Environment Research Agency (York, UK)	284.5	(248.3–318.9)
NGY	1997	Tobacco warehouse in Aichi, Japan	298.1	(264.9–331.0)
IWT	1999	Tobacco warehouse in Shizuoka, Japan	340.2	(305.7–375.1)
SKG	2010	Tobacco warehouse in Fukushima, Japan	412.6	(333.1–474.1)
MLY	2012	Tobacco warehouse in Shah Alam, Malaysia	1215.5	(1051.3–1351.6)

a Phosphine concentrations required to achieve 50% lethality (LC_{50}) for eggs at 72-h exposure, 25°C, and 75% r.h.

b. Insecticides

The efficacy of five commercial formulations of insecticide from three mode-of-action classes (IRAC, 2017) was evaluated: fenitrothion (Sumithion EC; Sumitomo Chemical Co., Ltd., Tokyo, Japan) and pirimiphos-methyl (Actellic EC; Nihon Nohyaku Co., Ltd., Tokyo, Japan) belonging to IRAC group 1B (organophosphates: acetylcholinesterase inhibitors); permethrin (Adion EC; Sumitomo Chemical

Co., Ltd., Tokyo, Japan) and bifenthrin (Talstar FL; Ishihara Sangyo Kaisha, Ltd., Osaka, Japan) belonging to the group 3A (synthetic pyrethroids: sodium channel modulators); and spinosad (Spinoace SC; Dow AgroSciences Japan, Ltd., Tokyo, Japan) belonging to the group 5 (nicotinic acetylcholine receptor allosteric modulators), all of which belong to different classes from phosphine (group 24A: mitochondrial complex IV electron transport inhibitors).

c. Insecticide susceptibility testing

Insecticide susceptibility was evaluated using dipping method. Thirty adults (collected within 3 d of emergence) that had been anesthetized with CO_2 were put into a glass tube (⌀21 mm, height 25 mm); then both ends of the tube were closed with polyester gauze. The insects within the glass tube were dipped for 10 s in the insecticide solution, which had been adjusted to the appropriate concentration (up to 10000 ppm) with water containing Triton X-100 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at 0.02%. After removing free drops of the solution using filter paper, the insects were transferred into a polystyrene vial (⌀25 mm, 50 mm depth) and were maintained at 27°C and 60% r.h.

The mortality was detemined at 24 h after exposure for fentrothion, pirimiphos-methyl, bifenthrin, and spinosad or at 48 h for permethrin. The viability was assessed as a measure of locomotion (i.e., paralyzed adults were regarded as dead).

Data were subjected to a probit analysis using the PriProbit (ver. 1.63) computer program developed by SAKUMA (1998), which was downloaded from https://www.ars.usda.gov/pacific-west-area/parlier/sjvasc/cpq/docs/priprobit-download/. The concentrations to achieve 50% lethality (LC$_{50}$) and 99% lethality (LC$_{99}$) were determined.

3. Results

Organophosphates fenitrothion and pirimiphos-methyl exhibited excellent performance at lower concentrations (< 20 ppm in LC$_{99}$). The susceptibilities were almost equal between the strains, irrespective of phosphine resistance (within 2.2 fold in resistance ratios calculated based on LC$_{50}$) (Table 2). The spinosad effect was constant among strains, although it was inferior to the two organophosphates tested. However, results show great differences in susceptibility to the two pyrethroids among strains. Three strains C87, SKG, and MLY exhibited high resistance to both permethrin and bifenthrin: most insects survived exposure, even at the highest concentration tested: 10000 ppm. To the other strains, TSC, THR, NGY, and IWT, bifenthrin exhibited high efficacy (≤ 25 ppm in LC$_{99}$), although permethrin was less effective (235–384 ppm in LC$_{99}$).

Table 2 Insecticide concentrations necessary to achieve 50% lethality (LC$_{50}$) and 99% lethality (LC$_{99}$) and resistance ratios of adults of *Lasioderma serricorne* strains

Insecticide	Strain	LC$_{50}$ (95% CI[a]), ppm		LC$_{99}$ (95% CI[a]), ppm		Resistance ratio[b]
Fenitrothion	TSC	5.9	(5.4–6.3)	11.6	(10.1–14.3)	1
	THR	6.0	(5.6–6.5)	12.5	(10.8–15.6)	1.0
	C87	6.6	(6.1–7.0)	13.0	(11.3–16.1)	1.1
	NGY	7.6	(6.5–9.2)	17.0	(12.7–35.3)	1.3
	IWT	6.2	(5.8–6.6)	11.2	(9.8–13.6)	1.1
	SKG	7.0	(6.6–7.5)	12.7	(11.2–15.3)	1.2
	MLY	5.9	(5.4–6.3)	13.4	(11.4–17.1)	1.0
Pirimiphos-methyl	TSC	4.2	(3.6–4.8)	19.2	(13.8–32.7)	1.9
	THR	2.3	(2.0–2.6)	5.9	(4.9–8.0)	1.1
	C87	2.5	(2.2–2.8)	8.7	(6.9–12.4)	1.2
	NGY	2.4	(2.0–2.8)	7.3	(5.8–10.7)	1.1
	IWT	2.1	(1.8–2.4)	5.5	(4.5–7.8)	1
	SKG	3.8	(3.4–4.1)	9.6	(7.9–13.2)	1.8
	MLY	4.7	(4.2–5.2)	12.2	(9.8–18.0)	2.2
Permethrin	TSC	120.8	(107.4–136.8)	384.0	(294.1–592.7)	1.7
	THR	97.2	(87.8–107.4)	235.3	(191.1–334.8)	1.4

	C87	>10000^c		>10000^c		>144.8
	NGY	79.3	(67.5–91.0)	340.2	(249.3–577.2)	1.1
	IWT	69.0	(58.6–78.8)	258.0	(195.6–415.1)	1
	SKG	>10000^c		>10000^c		>144.8
	MLY	>10000^c		>10000^c		>144.8
Bifenthrin	TSC	6.2	(5.6–6.9)	14.7	(12.2–20.2)	1.1
	THR	5.9	(5.2–6.6)	15.3	(12.5–21.6)	1
	C87	>10000^c		>10000^c		>1696.5
	NGY	7.0	(6.1–7.9)	25.2	(19.9–36.5)	1.2
	IWT	6.3	(5.4–7.2)	22.9	(18.0–33.7)	1.1
	SKG	>10000^c		>10000^c		>1696.5
	MLY	>10000^c		>10000^c		>1696.5
Spinosad	TSC	130.2	(111.4–152.6)	731.9	(511.6–1278.3)	1.9
	THR	152.8	(130.9–180.3)	856.2	(589.1–1543.6)	2.3
	C87	117.9	(101.4–136.8)	581.2	(421.4–952.5)	1.8
	NGY	115.3	(98.1–135.4)	682.1	(476.2–1190.9)	1.7
	IWT	100.8	(85.0–118.6)	722.6	(490.4–1322.4)	1.5
	SKG	125.2	(104.5–150.4)	1007.1	(646.1–2052.0)	1.9
	MLY	67.0	(50.7–83.4)	904.5	(532.3–2263.9)	1

[a] 95% confidence interval.

[b] Resistance ratios were calculated using the respective LC_{50} values relative to the strain which exhibited the smallest LC_{50} value for the respective insecticides.

[c] Probit analyses were not applied because most insects survived exposure even at the highest concentration: 10000 ppm.

4. Discussion

Higher contact toxicities of fenitrothion and pirimiphos-methyl against the same phosphine-susceptible *L. serricorne* strain as that examined in this experiment have been reported already (Orui, 2004). They have been used practically as space-sprays and surface-sprays in tobacco warehouses in the past, but the position as tobacco protectants of organophosphates has been replaced completely by pyrethroids. The absence of cross-resistance with phosphine, as shown in this experiment, makes them ideal for resistance-management. The time might come to review their usefulness against these pests.

Spinosad, derived by fermentation of soil actinomycete, has attracted attention as a grain protectant for its reduced risk properties (Hertlein et al., 2011). However, it has not been applied to tobacco in practice because it requires higher doses to achieve sufficient effects (Branc et al., 2004; Hertlein et al., 2011; Flingelli, 2014). Lower insecticidal activity against *L. serricorne* adults was found using a dipping method in this study (581–1007 ppm in LC_{99}). The cost of spinosad is higher than those of general synthetic insecticides at present. Therefore, it is not practical for the control of *L. serricorne* for economic reasons, although no cross-resistance with phosphine is apparent.

Synthetic pyrethroids are widely used today as surface-spray agents in tobacco warehouses around the world. This study revealed the existence of permethrin resistance and bifenthrin resistance in three of six phosphine-resistant strains which have mutually different origins. Coexistence of deltamethrin-resistance and phosphine-resistance in *L. serricorne* was found also in a population collected in a tobacco warehouse in Germany (Flingelli, 2014). Apart from tobacco pests, the widespread development of pyrethroid resistance has come to pose an immense practical difficulty in many parts of the world (Liu, 2012). These findings suggest that resistance to synthetic pyrethroids has developed in many field populations of *L. serricorne* through long-term use. Therefore, they cannot be recommended today as insecticides of primary choice for *L. serricorne*.

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Rapid detection of phosphine resistance in the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrychidae) from China using ARMS-PCR

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Abstract

The lesser grain borer, *Rhyzopertha dominica* is one of the serious cosmopolitan stored grain pests worldwide. High phosphine resistant *R. dominica* has been reported in several countries. The evolution of strong phosphine resistance is a major challenge for continuous application of the fumigant. Rapid detection of phosphine resistance level is a prime key to implement an appropriate strategy for control the stored-product pests. Dihydrolipoamide dehydrogenase (DLD) is a key metabolic enzyme mediating the phosphine resistance in population o*f R. dominica*, *Tribolium castaneum* and *Caenorhabditis elegans*. Analysis of the DLD sequences deposited in GenBank revealed that the P45/49S mutation was the most common one in many PH3-resistant stored-product pest insects. This information now enables direct detection of resistance using molecular diagnosis in field populations. We herein propose a method for rapid detection of phosphine resistance in *R. dominica* according to P49S point mutation of the DLD gene. Our data provides evidence that the ARMS-PCR method can be used for early warning of phosphine resistance in *R. dominica i*n field conditions.