Resistance of stored-product insects to phosphine

Marco Aurélio Guerra Pimentel[(1)], Lêda Rita D'Antonino Faroni[(2)], Maurílio Duarte Batista[(2)] and Felipe Humberto da Silva[(2)]

[(1)]Universidade Federal de Viçosa (UFV), Departamento de Biologia Animal, Setor de Entomologia, Avenida P.H. Rolfs, s/nº, Campus Universitário, CEP 36570-000 Viçosa, MG. E-mail: marcoagp@gmail.com [(2)]UFV, Departamento de Engenharia Agrícola, Setor de Armazenamento. E-mail: lfaroni@ufv.br, mauriliodbatista@yahoo.com.br, felipehumberto@gmail.com

Abstract – The objectives of this work were to assess phosphine resistance in insect populations (*Tribolium castaneum*, *Rhyzopertha dominica*, *Sitophilus zeamais* and *Oryzaephilus surinamensis*) from different regions of Brazil and to verify if the prevailing mechanism of phosphine resistance in these populations involves reduced respiration rates. Sixteen populations of *T. castaneum*, 15 of *R. dominica*, 27 of *S. zeamais* and eight of *O. surinamensis* were collected from 36 locations over seven Brazilian states. Each population was tested for resistance to phosphine, based on the response of adults to discriminating concentrations, according to FAO standard method. For each insect species, the production of carbon dioxide of the most resistant and of the most susceptible populations was inversely related to their phosphine resistance. The screening tests identified possible phosphine resistant populations. *R. dominica* and *O. surinamensis* were less susceptible to phosphine than the other two species. The populations with lower respiration rate showed a lower mortality at discriminating concentration, possibly related to a phosphine resistance mechanism. Phosphine resistance occurs in stored-product insects, in different regions of Brazil, and the resistance mechanism involves reduced respiration rate.

Index terms: *Oryzaephilus surinamensis*, *Rhyzopertha dominica*, *Sitophilus zeamais*, *Tribolium castaneum*, fumigant resistance, respiration rate.

Resistência de insetos de produtos armazenados à fosfina

Resumo – Os objetivos deste trabalho foram avaliar a resistência à fosfina, em populações de insetos (*Tribolium castaneum*, *Rhyzopertha dominica*, *Sitophilus zeamais* e *Oryzaephilus surinamensis*), de diferentes regiões do Brasil, e verificar se o mecanismo predominante de resistência à fosfina, nessas populações, envolve a redução das taxas respiratórias. Dezesseis populações de *T. castaneum*, 15 de *R. dominica*, 27 de *S. zeamais* e oito de *O. surinamensis* foram coletadas em 36 locais de sete estados brasileiros. Cada população foi testada quanto à resistência à fosfina, com base na resposta dos adultos à concentração discriminante, de acordo com o método padrão da FAO. Para cada espécie de insetos, as produções de dióxido de carbono da população mais resistente e a da mais suscetível foram inversamente relacionadas à resistência à fosfina. Os testes de detecção identificaram possíveis populações resistentes à fosfina. *R. dominica* e *O. surinamensis* foram menos suscetíveis à fosfina do que as outras duas espécies. As populações com menor taxa respiratória apresentaram menor mortalidade à concentração discriminante, o que está possivelmente relacionado ao mecanismo de resistência à fosfina. A resistência à fosfina ocorre em insetos de produtos armazenados, em diferentes regiões do Brasil, e o mecanismo de resistência envolve a redução da taxa respiratória.

Termos para indexação: *Oryzaephilus surinamensis*, *Rhyzopertha dominica*, *Sitophilus zeamais*, *Tribolium castaneum*, resistência a fumigante, taxa respiratória.

Introduction

Phosphine is the primary fumigant used to protect the majority of the world's grain and a variety of other stored commodities against insect pests (Daglish, 2004; Collins et al., 2005). Phosphine plays an important role for protection especially because of its low cost, fast dispersion in the air, low residues, and human intolerance to contact pesticides. These advantages have contributed to increased dependence on this fumigant (Chaudhry, 2000; Hasan & Reichmuth, 2004; Wang et al., 2006). However, long-term use of a single fumigant also increases the risk of appearance of resistant pest populations (Bengston et al., 1999; Collins et al., 2002; Daglish, 2004).

Insect resistance to fumigants used for stored products is problematic throughout the world (Champ & Dyte, 1976; Benhalima et al., 2004; Collins et al., 2005; Wang et al., 2006). In recent years, the importance of phosphine to protect stored grain products has grown due to international agreements phasing out the fumigant methyl bromide (Bell, 2000; Zettler & Arthur, 2000; Rajendran, 2001). In addition, the lack of ideal airtight conditions for fumigation, in most storage units, increases the frequency of control failures and, consequently, increases the frequency of applications (Pacheco et al., 1990; Chaudhry, 2000; Benhalima et al., 2004; Lorini et al., 2007). These factors combine to result in higher selection pressure for phosphine resistance (Bengston et al., 1999; Collins et al., 2002).

A 1972–1973 global survey by the Food and Agriculture Organization (FAO) showed that about 10% of sampled insect populations worldwide contained phosphine resistant individuals, except for Brazil (Champ & Dyte, 1976). Since FAO's global report, there have been few surveys for phosphine resistance of stored-product insects in Brazil. There are only three reports showing phosphine resistance in Brazilian populations of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) (Pacheco et al., 1990; Sartori et al., 1990; Lorini et al., 2007). The current levels of phosphine resistance in Brazilian populations of *T. castaneum*, *R. dominica*, *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) are not known.

Since resistance to phosphine is increasing among insect pests, improved monitoring and management of resistance are priorities. The understanding of phosphine resistance mechanism in these insects is essential for their management, and may also greatly reduce the efforts and expenses for identifying synergists or novel replacement compounds (Bell, 2000; Zettler & Arthur, 2000; Rajendran, 2001; Chaudhry et al., 2004). The most accepted mechanism is the reduced uptake of the fumigant (Price, 1984), which may be closely linked to insect's respiration rate, and is generally overlooked. Respiration is also a good indicator of insect's physiological response to the environment to which they are exposed (Chaudhry, 2000; Emekci et al., 2002, 2004; Chaudhry et al., 2004).

Monitoring changes in the susceptibility of insects are essential to manage phosphine resistance (Collins et al., 2002, 2005). Through such monitoring, it is possible to evaluate the efficiency of commonly used management strategies to delay resistance development. Studies regarding phosphine resistance management are of particular interest to control resistant populations (Haubruge & Arnaud, 2001).

The objectives of this work were to assess phosphine resistance in insect populations (*Tribolium castaneum*, *Rhyzopertha dominica*, *Sitophilus zeamais* and *Oryzaephilus surinamensis*) from different regions of Brazil and to verify if the prevailing mechanism of phosphine resistance in these populations involves reduced respiration rates.

Materials and Methods

The experiments were carried out at Laboratório de Manejo Integrado de Pragas de Grãos Armazenados, Universidade Federal de Viçosa, Viçosa, MG, Brazil, from January to November 2007.

Sixteen populations of *T. castaneum*, 15 of *R. dominica*, 27 of *S. zeamais* and eight of *O. surinamensis* were collected from 36 locations over seven states of Brazil (Table 1). The insects were reared in 1.5 L glass bottles at 28±1°C and 70±2% air relative humidity. Broken corn kernels were used as food for *T. castaneum* and *O. surinamensis*, whole corn kernels for *S. zeamais*, and whole wheat kernels for *R. dominica* (13% moisture content). To avoid field cross-infestation, kernels were stored at -18°C for 96 hours for disinfestation prior to use.

Discriminating concentrations recommended by FAO (FAO, 1975) were used to detect phosphine resistance in adult insects (0.03 mg L^{-1} for *R. dominica*, 0.04 mg L^{-1} for *T. castaneum* and 0.05 mg L^{-1} for *O. surinamensis* and *S. zeamais*); the fumigation was done at 25°C and 70% air relative humidity. The phosphine concentration in the source (phosphine generator) was checked before bioassays.

Phosphine (about 86% purity) was produced using 0.6 g aluminum phosphide tablets in acidified water (sulfuric acid 5%). Adult beetles (1–3 weeks old males and females) were confined in ventilated plastic containers, held in a gas-tight desiccator. There were 50 adults (males and females) per container and two containers per desiccator. The required amount of phosphine was injected by a gas-tight syringe through a septum in the lid of the desiccator. After fumigation, the containers were removed from the desiccators and kept for 14 days at 25°C and 70% air relative humidity, before assessing the mortality. Corrected mortality was calculated as follows: corrected mortality = 100[(mortality in treated insects - mortality in control insects)/(100 - mortality in control insects)] (Abbott, 1925). The experiment was carried out in a complete randomized design, with four replicates for each population.

The carbon dioxide (CO_2) produced by the insects of most resistant and most susceptible populations of each species was measured in a CO_2 analyzer (TR 2 Sable System International), based on a modified method of Guedes et al. (2006). A series of 25 mL flasks, containing 20 insects of a population each, were placed in a completely hermetic system, and CO_2 production in each flask was measured at 25°C, after an acclimatizing period of 5 hours. Three replicates were used for each population. CO_2-free air was fluxed for 2 min through the flasks, at 600 mL flow rate, and the infrared detector was connected to the outlet of the system to quantify CO_2 (μmol per insect per hour).

The corrected mortalities were submitted to analysis of variance and the means were compared by Tukey's test, at 5% of probability, using PROC GLM (SAS Institute, 1999). The respiration rate (CO_2 production) of the resistant and susceptible populations of each species was submitted to t test, to verify significant variation in respiration rate among populations, at 5% of probability, using PROC TTEST (SAS Institute, 1999).

Table 1. Local and period of sampling of Brazilian populations of *Tribolium castaneum*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis* and *Sitophilus zeamais*. The insect populations are identified by the Brazilian county of origin and its respective State.

County and state	Sampling site	Month/year	Species[(1)]
Abre Campo I, MG	Metallic bin	November/2005	S
Abre Campo II, MG	Metallic bin	November/2005	S
Água Boa, MT	Warehouse	August/2004	T/R
Aguanil, MG	Farm wood store	May/2005	T
Alfenas, MG	Metallic bin	April/2004	T
Anápolis I, GO	Metallic bin	August/2007	S
Anápolis II, GO	Metallic bin	August/2007	S
Astolfo Dutra, MG	Industry of foods	September/2005	O
Bom Despacho, MG	Metallic bin	May/2005	T/R/O
Bragança Paulista, SP	Farm wood store	March/2005	T
Cambé, PR	Metallic bin	December/2005	R
Campos de Júlio, MT	Metallic bin	June/2004	T/R
Canarana, MT	Warehouse	January/2005	S
Carmo do Rio Claro, MG	Metallic bin	August/2005	R
Coimbra, MG	Field strain	April/2007	S
Cristalina, GO	Warehouse	December/2006	R/S
Frutal, MG	Metallic bin	December/2005	T
Guarapuava I, PR	Metallic bin	August/2006	S
Guarapuava II, PR	Metallic bin	August/2006	S
Guaxupé I, MG	Metallic bin	August/2005	O/S
Guaxupé II, MG	Metallic bin	August/2005	S
Iporá, GO	Warehouse	December/2006	R/S
Jacarezinho, PR	Laboratory	-	S
Jacuí, MG	Farm wood store	April/2005	T/S
Jataí, GO	Metallic bin	September/2006	S
Machado, MG	Industry of Foods	July/2005	S
Nova Era, MG	Warehouse	May/2005	T/R/S
Nova Xavantina, MT	Warehouse	May/2007	R
Palmital, SP	Metallic bin	March/2005	R
Paracatu, MG	Metallic bin	December/2006	S
Picos, PI	Farm wood store	April/2007	T/S
Piracicaba, SP	Laboratory	Agosto/2004	T/R/S
Rio Verde, GO	Metallic bin	July/2004	T/R/S
Sacramento, MG	Warehouse	May/2005	O/S
São Miguel do Anta, MG	Farm wood store	July/2007	S
Tunápolis, SC	Farm wood store	May/2005	S
Uberlândia, MG	Metallic bin	August/2004	T/R/O
Unaí I, MG	Metallic bin	August/2004	T/R/O/S
Unaí II, MG	Metallic bin	July/2004	T/O/S
Viçosa, MG	Laboratory	March/2004	T/R/O/S

[(1)]O: *O. surinamensis*; R: *R. dominica*; S: *S. zeamais*; T: *T. castaneum*.

Results and Discussion

The screening test using discriminating concentration of phosphine was useful to identify the resistant populations (Table 2). The mortality at the discriminating concentration varied significantly among populations of *T. castaneum* ($p<0.0001$), *R. dominica* ($p<0.0001$), *O. surinamensis* ($p<0.0001$) and *S. zeamais* ($p<0.0001$). There was a considerable intrapopulation variation in susceptibility for the species studied. The mortality of *R. dominica* and *O. surinamensis* populations did not exceed 80%, and some populations of *S. zeamais* and one population of *T. castaneum* had 100% mortality. The corrected mortality (smaller than 30% mortality) was used to identify a possible resistant population for each of the four species, as well as susceptible populations.

The use of the discriminating concentration for early monitoring of phosphine resistance in wild populations of stored-product beetles is a valuable tool, because this concentration is expected to kill all susceptible specimens. If entire test population dies at the end of the post-treatment holding period, the sample can be classified as having "no detectable resistance", while the presence of a single unaffected insect should be considered as first evidence of resistance, requiring further investigation (Champ & Dyte, 1976).

The number of insects surviving at the discriminating concentration in a population sample will indicate the frequency of resistant individuals. At the discriminating concentration, 100% survival was observed for: *T. castenum* populations from Uberlândia, Rio Verde and Bom Despacho counties; *R. dominica* populations from Uberlândia, Bom Despacho, and Palmital; and populations of *O. surinamensis* from Guaxupé and Astolfo Dutra counties (Table 2). Out of 40 samples collected between 2004 and 2007, 45% were resistant to phosphine and 35% presented more than 90% survival at the discriminating concentration.

These results clearly demonstrate that resistance to phosphine in the four species has become widespread in these important grain-growing regions of Brazil. Previous surveys pointed out that phosphine resistance was, at the time, a rare event, and resistance levels were low (Champ & Dyte, 1976; Pacheco et al., 1990; Sartori et al., 1990). The present work reports for the first time the existence of phosphine resistance in stored-product beetles in the states of Mato Grosso and Minas Gerais, and it is also the first report on phosphine resistance in populations of *O. surinamensis* in Brazil. This information is crucial for managing resistance to phosphine in Brazil, because of the widespread resistance detected in new agricultural areas.

Despite the high level of resistance reported in this work (Table 2), absence of phosphine resistance in some populations of *S. zeamais* and in one population of *T. castaneum* is not surprising

Table 2. Mortality at the discriminating concentration of phosphine for populations of *Tribolium castaneum*, *Sitophilus zeamais*, *Rhyzopertha dominica* and *Oryzaephilus surinamensis* collected from different regions of Brazil[(1)].

Population	Mortality at discriminating concentration (%)			
	Tribolium castaneum	*Sitophilus zeamais*	*Rhyzopertha dominica*	*Oryzaephilus surinamensis*
Jacuí	100.00±0.00a	100.00±0.00a	-[(2)]	-
Unaí II	90.15±8.70ab	8.95±1.77e	-	10.81±0.14b
Água Boa	89.50±7.90ab	-	32.50±9.98bcd	-
Aguanil	79.85±3.91b	-	-	-
Bragança Paulista	59.94±7.87c	-	-	-
Piracicaba	57.65±8.33c	100.00±0.00a	24.00±14.14cd	-
Picos	36.70±2.94d	100.00±0.00a	-	-
Unaí	11.54±6.30e	27.29±1.82d	23.00±12.73cd	72.20±7.41a
Viçosa	6.50±7.90e	98.02±0.03a	21.00±7.75de	3.31±1.31b
Frutal	5.39±5.44e	-	-	-
Nova Era	4.83±1.17e	100.00±0.00a	12.00±0.00de	-
Alfenas	4.48±3.36e	-	-	-
Campos de Júlio	3.00±1.41e	-	44.00±3.65bc	-
Uberlândia	0.00±0.00e	-	0.00±0.00e	14.44±5.38b
Rio Verde	0.00±0.00e	100.00±0.00a	23.00±1.41cd	-
Bom Despacho	0.00±0.00e	-	0.00±0.00e	4.09±0.12b
Carmo do Rio Claro	-	-	77.50±3.54a	-
Nova Xavantina	-	-	42.95±11.24bc	-
Cambé	-	-	20.20±3.11de	-
Palmital	-	-	0.00±0.00e	-
Sacramento	-	100.00±0.00a	-	6.37±5.11b
Guaxupé I	-	100.00±0.00a	-	0.00±0.00b
Guaxupé II	-	96.82±1.05a	-	-
Astolfo Dutra	-	-	-	0.00±0.00b
Abre Campo I	-	98.96±1.47a	-	-
Abre Campo II	-	99.00±1.41a	-	-
Tunápolis	-	100.00±0.00a	-	-
Canarana	-	100.00±0.00a	-	-
Jataí	-	98.12±2.67a	-	-
Cristalina	-	75.74±3.20b	32.30±2.40bcd	-
Guarapuava I	-	90.28±2.88a	-	-
Guarapuava II	-	54.41±6.24c	-	-
Machado	-	9.00±3.90e	-	-
Jacarezinho	-	99.00±1.41a	-	-
Coimbra	-	100.00±0.00a	-	-
Iporá	-	93.87±3.52a	52.05±8.84b	-
São Miguel do Anta	-	92.16±0.00a	-	-
Anápolis I	-	94.12±8.32a	-	-
Anápolis II	-	97.87±3.01a	-	-
Paracatu	-	21.38±3.71d	-	-

[(1)]Means±SE followed by the same letters in columns do not differ significantly by Tukey's test, at 5% of probability. [(2)]Non-tested populations.

(Benhalima et al., 2004; Wang et al., 2006). Also, due to poor fumigation standards (Lorini et al., 2007), phosphine concentration does not reach the level required for selection of resistance at immature stages (Bengston et al., 1999; Collins et al., 2002, 2005; Benhalima et al., 2004).

The phosphine resistance detected among the populations may be the result of previous selection pressure caused by inadequate fumigations in the storage units, from where the samples were taken. Other reasons for differences in phosphine resistance observed in the present work were: high insect populations favored by climate; little or no insect control in storage units in some farms (Collins et al., 2005; Lorini et al., 2007); problems of widespread resistance to grain protectants (Lorini & Galley, 1999); storage facilities not adequately sealed before fumigation; and fumigant concentrations not being monitored (Benhalima et al., 2004; Wang et al., 2006; Lorini et al., 2007). As consequence, some lots of grains were fumigated frequently, and since the populations did not respond to the control, very high doses of phosphine were used, increasing problems for selection for resistance (Collins et al., 2005; Lorini et al., 2007).

The resistant and susceptible populations used for respiratory assays were collected, respectively, from: Bom Despacho and Jacuí for *T. castaneum*; Uberlândia and Carmo do Rio Claro for *R. dominica*; Guaxupé and Unaí for *O. surinamensis*; and Unaí II and Piracicaba for *S. zeamais*. The production of CO_2 varied significantly among populations of *T. castaneum* ($p<0.001$), *R. dominica* ($p<0.001$), *O. surinamensis* ($p<0.001$) and *S. zeamais* ($p<0.030$) (Table 3). For the four species, the populations with the highest respiration rate (CO_2 production) had the highest mortality at discriminating phosphine concentration.

Table 3. Respiration rate (CO_2 production) of phosphine-susceptible and resistant populations of *Tribolium castaneum*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis* and *Sitophilus zeamais*.

Species	Populations/resistance status[1]	Respiration rate (μmol CO_2 per insect per hour)
T. castaneum	Bom Despacho/R	0.0111±0.0004
	Jacuí/S	0.0818±0.0042
R. dominica	Uberlândia/R	0.0575±0.0024
	Carmo do Rio Claro/S	0.2068±0.0446
O. surinamensis	Guaxupé/R	0.0215±0.0017
	Unaí/S	0.0365±0.0019
S. zeamais	Unaí II/R	0.3439±0.0217
	Piracicaba/S	0.4718±0.0107

[1]Resistance status: R, phosphine resistant; S, phosphine susceptible.

The results of the respiratory assays support the hypothesis that low respiration rates (CO_2 production) are correlated to high resistance levels, because low respiration results in reduced uptake of the fumigant. This hypothesis was confirmed in studies using radioactive fumigants (Chaudhry et al., 2004) and was also demonstrated in *R. dominica* (Price, 1984) and *T. castaneum* (Nakakita & Kuroda, 1986). The exposure of some populations of *R. dominica*, *S. oryzae* and *T. castaneum* to [^{32}P]-labelled phosphine showed that [^{32}P] uptake by resistant strains of all the three species was much lower than that by susceptible strains (Benhalima et al., 2004). Under similar conditions of exposure (0.7 g m^{-3} of [^{32}P]H_3 for 5 hours at 25°C), a susceptible strain of *T. castaneum* absorbed seven times more gas per gram of insect than a resistant strain. Moreover, the present results suggest that the lower phosphine uptake in phosphine-resistant populations of stored-product insect species may have occurred and may have been caused by the reduction in the respiration rate.

There is an urgent need to change fumigation and pest management practices in Brazil, to manage for phosphine resistance. Further work is required to confirm what strains are resistant to phosphine, to determine strategies to manage resistance to phosphine, and to develop alternative fumigants.

Conclusions

1. Many of the insect populations tested have high frequencies of phosphine resistant individuals.
2. Phosphine resistance occurs in stored-product insects from all seven Brazilian states where samples were collected.
3. The mortality rate of selected resistant populations, in relation to selected susceptible ones, is inversely related to their respiration rate.

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