PEST MANAGEMENT

Spread of Phosphine Resistance among Brazilian Populations of Three Species of Stored Product Insects

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ABSTRACT - The resistance to fumigant insecticides in stored-products insects is often recorded. Several factors influence the evolution of insecticide resistance. Among these, the frequency of applications and the migration of resistant populations are of primary importance for the stored-product insects. The aim of this study was to characterize the spectrum and investigate the status of phosphine resistance in Brazil, in 13 populations of the Coleoptera *Tribolium castaneum* Herbst (Tenebrionidae), ten populations of *Rhyzopertha dominica* (Fabr.) (Bostrichidae), and eight populations of *Oryzaephilus surinamensis* (L.) (Silvanidae). The pattern of resistance dispersion in the populations of these species was also verified. The bioassays for the detection of phosphine resistance followed the FAO standard method. To test the influence of migration in the evolution of the phosphine resistance, the difference of mortality in the discriminating concentration and the geographical distance among each pair wise combination of collection sites were correlated. None of the populations exhibited mortality above 90% in the discriminating concentration, for the three species. Mortality in the discriminating concentration increased with the geographical distance for *R. dominica* and *O. surinamensis*. However, no significant linear response was observed among the variables for *T. castaneum* populations. These results suggest that the dispersion of insects and the local selection are relevant in the evolution of the phosphine resistance in populations of *R. dominica* and *O. surinamensis*. In contrast, grain trade and local selection are probably the factors that determine the evolution of the phosphine resistance in populations of *T. castaneum*.

KEY WORDS: *Tribolium castaneum*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis*, insecticide resistance, migration, stored grain

Insects are a serious problem in storage products, particularly in the tropics. The relative importance of the different insect pest species of stored products varies among areas and depends on the stored product (Champ & Dyte 1976). In Brazil, some species prevail due to their high reproduction capacity, broad distribution and high level of acquired resistance to the fumigants and contact insecticides (Pacheco *et al* 1990, Sartori *et al* 1990, Guedes *et al* 1995, 1996, Lorini & Galley 1999).

The red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), the lesser grain borer, *Rhyzopertha dominica* (Fabr.) (Coleoptera: Bostrichidae), and the sawthoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), are the main cosmopolitan pests of stored cereals in the tropics, together with the cereal weevils *Sitophilus* spp. (Coleoptera: Curculionidae). The main method for controlling stored-product insects in warm climates is the use of insecticides due to the lack of other control alternatives (Guedes 1991). However, insecticide resistance is evident in many field populations of insects that infest stored products, where these compounds are extensively used (Champ & Dyte 1976, Badmin 1990, Pacheco *et al* 1990, Guedes *et al* 1995, 1996, 1997bc, 1998, Subramanyam & Hagstrum 1996).

Phosphine has been widely used as fumigant for the control of stored-product insects for almost half century (Price & Mills 1988, Chaudhry 2000). It is by far the most widely used fumigant because of its low cost, fast diffusion in the air, and absence of detectable residue levels (Chaudhry 2000). These advantages contributed to an increased dependence on phosphine as fumigant (Chaudhry 2000). However, the long-term use of a single fumigant increases the risk of phosphine resistance development in pest populations (Benhalima *et al* 2004).

Resistance to fumigants is an increasing problem in controlling stored-product insects throughout the world (Champ & Dyte 1976, Price & Mills 1988). The importance of phosphine to protect stored grain products has recently increased even further due to international agreements to phase out the use of the fumigant methyl bromide (UNEP

1995). In addition, the lack of airtight conditions for fumigation in most storage units increases the frequency of control failures and consequently increases the frequency of applications (Tyler *et al* 1983, Pacheco *et al* 1990, Chaudhry 2000, Benhalima *et al* 2004). This results in an increased selection pressure for phosphine resistance (Chaudhry 1997, 2000).

A global survey undertaken by the Food and Agriculture Organization (FAO) in 1972/1973 showed that about 10 % of the collected populations contained phosphine resistant individuals, but phosphine resistance was not found in Brazil at that time (Champ & Dyte 1976). Since this survey, many control failures with phosphine have been reported in Brazil, allegedly due to resistance (Pacheco *et al* 1990, Sartori *et al* 1990). Moreover, phosphine resistance in stored-product insects is documented in several neighboring South American countries (Champ & Dyte 1976, Pacheco *et al* 1990, Sartori *et al* 1990). Except for the global survey undertaken by FAO (Champ & Dyte 1976), surveys for phosphine resistance of stored-products insects in Brazil are rare. There are only five reports showing phosphine resistance in Brazilian populations of *R. dominica*, *T. castaneum*, *Sitophilus oryzae* (L.), *Sitophilus zeamais* Mots. and *Cryptolestes ferrugineus* (Stephens) (Pacheco *et al* 1990, Sartori *et al* 1990, Lorini *et al* 2007, Pimentel *et al* 2007, 2009).

Several factors influence the evolution of the resistance to insecticides (Georghiou 1972, Roush & Daly 1990, Mallet 1993, Hoy *et al* 1998). Among these, the frequency of insecticide applications and migration of resistant populations are of primary importance for the stored-product insects. Studies on migration of resistant populations of stored-products insects are scarce, except for an earlier initial study with populations of *R. dominica* and a subsequent study with organophosphate and pyrethroid resistant strains of *S. zeamais* where the lack of correlation between geographic distance and the resistance status suggests that local selection and/or broad dispersal of resistant populations by grain trade are major factors in the evolution of insecticide resistance in populations of this species, a contrast with the findings reported for the coffee leaf miner *Leucoptera coffeella* (Guérin-Menéville) (Lepidoptera: Lyonetiidae) (Guedes *et al* 1997a, Fragoso *et al* 2003ab).

The understanding of the dispersion patterns of phosphine resistant populations of stored-product insects is fundamental to phosphine resistance management. Therefore, the aim of our study was (i) to characterize the spectrum of resistance and investigate the status of phosphine resistance in Brazilian populations of *T. castaneum*, *R. dominica,* and *O. surinamensis*; and (ii) to verify the pattern of resistance dispersion in the populations of these species.

Material and Methods

Insects. Thirteen populations of *T. castaneum*, ten of *R. dominica* and eight of *O. surinamensis* collected at sixteen sites in four Brazilian states were used in this study (Fig 1, Table 1). Nine of them were field-collected between March and August 2004 and eight of them were collected between March and September 2005 and at least 400 individuals were used to establish each population in the laboratory. These populations were reared in glass jars (1.5 l) and

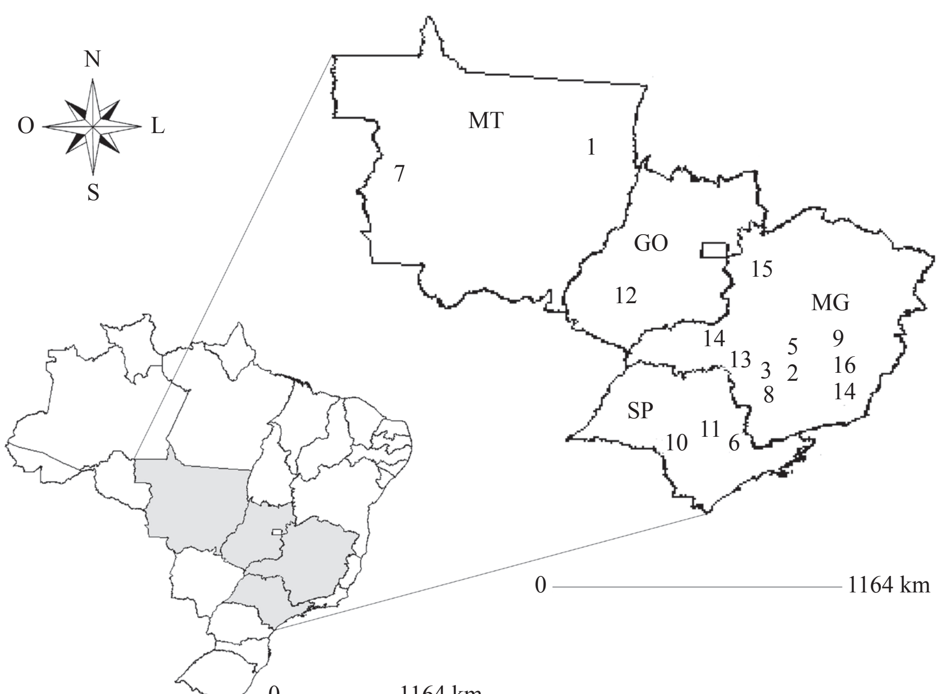

Fig 1 Map of Brazil with the states of Goiás (GO), Mato Grosso (MT), Minas Gerais (MG), and São Paulo (SP) in evidence showing the sampling sites for *Tribolium castaneum*, *Rhyzopertha dominica,* and *Oryzaephilus surinamensis*. The numbers correspond to locations in Table 1.

Table 1 Codes, origin, time and place of collection of Brazilian populations of *Tribolium castaneum* (T), *Rhyzopertha dominica* (R) and *Oryzaephilus surinamensis* (O).

Code	County	State	Facility	Time	Species
1	Água Boa	MT	Warehouse	August 2004	T and R
2	Aguanil	MG	Farm wood store	May 2005	T
3	Alfenas	MG	Metallic bin	April 2004	T
4	Astolfo Dutra	MG	Industry of Foods	September 2005	O
5	Bom Despacho	MG	Metallic bin	May 2005	T, R, and O
6	Bragança Paulista	SP	Farm wood store	March 2005	T
7	Campos de Júlio	MT	Metallic bin	June 2004	T and R
8	Guaxupé	MG	Metallic bin	August 2005	O
9	Nova Era	MG	Warehouse	May 2005	T and R
10	Palmital	SP	Metallic bin	March 2005	R
11	Piracicaba	SP	Laboratory	August 2004	T and R
12	Rio Verde	GO	Metallic bin	July 2004	T and R
13	Sacramento	MG	Warehouse	May 2005	O
14	Uberlândia	MG	Metallic bin	August 2004	T, R, and O
15a	Unaí	MG	Metallic bin	August 2004	T, R, and O
15b	Unaí II	MG	Metallic bin	July 2004	T and O
16	Viçosa	MG	Laboratory	March 2004	T, R, and O

maintained under controlled conditions of 28 ± 2ºC and RH: 70 ±5%. Broken corn grains were used as food source for *T. castaneum* and *O. surinamensis* and whole wheat grains were used for *R. dominica* (13% moisture content). Grains were previously disinfected and kept at -18ºC to avoid field cross-infestation.

Bioassays of phosphine resistance. Discriminating concentrations recommended by FAO (FAO 1975) were used for detecting 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*). Fumigation of adults was based on the FAO method (FAO 1975) and took place at 25ºC and RH: 70%. The concentration of the phosphine source was always checked before the bioassays.

Phosphine (*ca*. 86% pure) was produced using aluminum phosphide tablets (0.6 g) in acidified water (sulfuric acid 5%). Adult beetles (2-4 weeks old) were confined in ventilated plastic containers inside gas-tight desiccators. Fifty unsexed adults were placed in each container and two containers were placed in each desiccator. Phosphine was injected with gas-tight syringes through a septum in the lid of each desiccator to produce the required concentration. After fumigation (20h period of exposure), the containers were removed from the desiccators and kept for 14 days (25°C and RH: 70%), after which mortality was assessed. Natural mortality was corrected following Abbott (1925).

Resistance dispersal. The influence of the migration in the evolution of the phosphine resistance in the stored-product insects was tested correlating phosphine resistance and distance between sampling sites. The geographical coordinates of the population sampling sites were used to calculate the geographical distances between any two sites obtained by global positioning satellite (Garmin GPS 12XL; Olathe, KS, USA). The pair wise differences of mortality at discriminating concentration of phosphine were correlated with the respective geographical distance among the sites of collection of the populations.

Statistical analysis. The corrected mortality (%) was subjected to analysis of variance followed by Tukey's HSD test (P < 0.05, PROC GLM, SAS Institute 1989). The relationships between corrected mortality at discriminating concentration (%) and the respective geographical distance (km) among the places of origin of the populations were subjected to regression analysis for the three insect species (PROC GLM, SAS Institute 1989).

Results

Phosphine resistance. The mortality at discriminating concentration varied significantly among populations of *T. castaneum* ($F_{12,43}$ = 133.99, P < 0.0001), *R. dominica* ($F_{9,31}$ = 21.31, P <0.0001) and *O. surinamensis* ($F_{7,22}$ = 94.92, P < 0.0001) (Table 2). None of the populations surveyed showed mortality superior to 90%, for all of the three species. The number of insects that survived the discriminating concentration of phosphine further indicated that some of the population samples tested had a high frequency of resistant individuals. For example, the *T. castaneum* populations of Uberlândia, Rio Verde and Bom Despacho showed 100% of survival at the discriminating concentration of phosphine.

Table 2 The response of field populations of *Tribolium castaneum*, *Rhyzopertha dominica,* and *Oryzaephilus surinamensis* collected in Brazil to the discriminating concentration of phosphine

Populations	Mortality at discriminating concentration (%) ± S.E.M.		
	T. castaneum	*R. dominica*	*O. surinamensis*
Unaí II	90.1 ± 8.70 a*	-	10.8 ± 0.14 b
Água Boa	89.5 ± 7.90 a	32.5 ± 9.98 ab	-
Aguanil	79.8 ± 3.91 a	-	-
Bragança Paulista	59.9 ± 7.87 b	-	-
Piracicaba	57.6 ± 8.33 b	24.0 ± 14.14 bc	-
Unaí	11.5 ± 6.29 c	23.0 ± 12.73 bc	72.2 ± 7.41 a
Viçosa	6.5 ± 7.90 c	21.0 ± 7.75 bc	3.3 ± 1.31 b
Nova Era	4.8 ± 1.17 c	12.0 ± 0.00 cd	-
Alfenas	4.5 ± 3.36 c	-	-
Campos de Júlio	3.0 ± 1.41 c	44.0 ± 3.65 a	-
Uberlândia	0.0 ± 0.00 c	0.0 ± 0.00 d	14.4 ± 5.38 b
Rio Verde	0.0 ± 0.00 c	23.0 ± 1.41 bc	-
Bom Despacho	0.0 ± 0.00 c	0.0 ± 0.00 d	4.1 ± 0.12 b
Palmital	-	0.0 ± 0.00 d	-
Sacramento	-	-	6.4 ± 5.11 b
Guaxupé	-	-	0.0 ± 0.00 b
Astolfo Dutra	-	-	0.0 ± 0.00 b

*Means followed by the same letter in a column are not significantly different by Tukey's HSD multiple range test (P < 0.05).

For *R. dominica*, the populations of Uberlândia, Bom Despacho and Palmital showed 100% of survival and for *O. surinamensis* the populations of Guaxupé and Astolfo Dutra present also 100% of survival at the discriminating concentration of phosphine (Table 2).

Resistance dispersal. There was no significant ($F_{1,131}$ = 0.11; P < 0.7373) linear response between resistance and dispersal for the populations of *T. castaneum*, however the correlation between these variables was significant for the populations of *R. dominica* ($F_{1,89}$ = 22.00; P < 0.0001) and *O. surinamensis* ($F_{1,41}$ = 13.29; P < 0.0008) (Fig 2). The mortality variation at the discriminating concentrations increased with the geographical distances for *R. dominica* and *O. surinamensis*. This finding suggests that the dispersion of insects is relevant in the evolution of resistance to phosphine, particularly for *R. dominica* and *O. surinamensis*. However, especially for stored-products insects, grain trade within the country, together with local selection, are probably the main factors determining the spread and evolution of phosphine resistance in Brazil, due to high turnover of grain from producing and consuming regions.

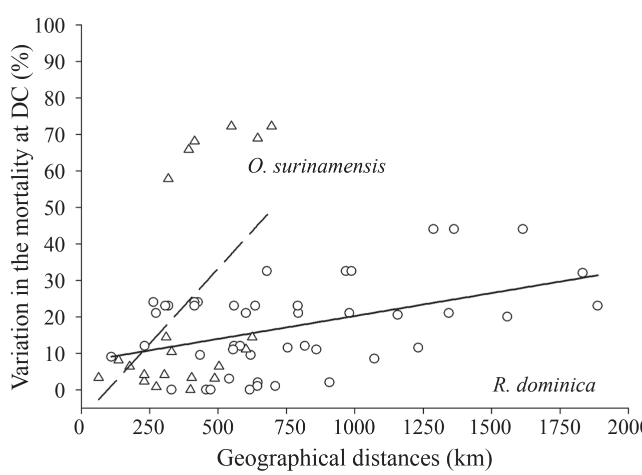

Fig 2 Variation in the mortality at discriminating concentration (%) of *Rhyzopertha dominica* (○) and *Oryzaephilus surinamensis* (Δ) as a function of geographical distances (km). Curves were fit using regression analyses for *R. dominica* (y = 7.64 + 0.01x; r^2 = 0.20; $F_{1,89}$ = 22.00; P < 0.0001) and *O. surinamensis* (y = -8.05 + 0.08x; r^2 = 0.25; $F_{1,41}$ = 13.29; P < 0.0008).

Discussion

The presence of unaffected insects at the end of the test in some populations should be regarded as evidence of resistance, requiring further investigation. However, the evidence of the occurrence of phosphine resistance, with different frequencies, in all populations of *T. castaneum*, *R. dominica* and *O. surinamensis* from the states of Goiás, Mato Grosso, Minas Gerais, and São Paulo are shown in the results of the present study (Table 2). This is the first report of phosphine resistance in stored product pests from Mato Grosso and Minas Gerais. Hence, phosphine resistance is widely spread in stored product insect-pests in Brazil.

Some populations of *T. castaneum* (Uberlândia-MG, Bom Despacho-MG, and Rio Verde-GO), *R. dominica* (Palmital-SP, Bom Despacho-MG, and Uberlândia-MG), and *O. surinamensis* (Astolfo Dutra-MG and Guaxupé-MG) showed 100% of survival at the FAO discriminating concentration (Table 2). These results were expected because more recent studies with Brazilian strains of *R. dominica* also found unaffected insects after bioassays with FAO discriminating concentration. For instance, Lorini *et al* (2007) in a study with 19 strains of *R. dominica* from Brazil found survivors at discriminating concentration. Of the 19 samples tested, five could be diagnosed with weak resistance and 14 with strong resistance suggesting that resistance was widespread and apparently at a high level. Therefore, our results are in accordance with earlier surveys of phosphine resistance in Brazil (Lorini *et al* 2007, Pimentel *et al* 2007, 2009) and also show evidence of resistance, but requiring further investigation for these species studied here.

This low mortality at the discriminating concentration may suggest that these populations have been under high selection pressure for many years (Benhalima *et al* 2004, Collins *et al* 2005). In addition, the frequent unsuitable storage management added to poor fumigant application techniques has probably enhanced the selection pressure for resistance (Sartori *et al* 1990, Lorini *et al* 2007, Pimentel *et al* 2007). Another factor that can influence the evolution of phosphine resistance is the movement of insects due to the commodity trade (Benhalima *et al* 2004, Fragoso *et al* 2003b).

Many species of stored-product insects are capable of long-distance flight, of breeding on many different wild hosts, and feeding on flowers and grass to increase adult longevity and fecundity (Hagstrum *et al* 1996, Jia *et al* 2008). Besides this, the several species of stored-product insects are known to infest crops in the field prior the harvest (Hagstrum 1985) and can be found within woodlands far from grain storage sites (Campbell *et al* 2006). Other studies reporting the distribution of insects inside structures (Campbell *et al* 2002), around outside of structures (Campbell & Mullen 2004) and sometimes far away from these structures (Sinclair & Haddrell 1985) suggest the capability for long distance flight, which is also confirmed by some laboratory experiments (Perez-Mendoza *et al* 1999a, b).

Our results with populations of *R. dominica* and *O. surinamensis*¸ but not *T. castaneum*, provide support for this assumption. The lack of spatial pattern was evidenced by the lack of correlation between geographical distance and mortality at discriminating concentration spectrum for each population of *T. castaneum*, as also reported for *S. zeamais* and contrary to what was observed for at least one tropical pest species, the coffee leaf miner (Fragoso *et al* 2003ab). Our results for *T. castaneum* suggest that local selection and/or broad dispersal of resistant populations by grain trade are probably the major forces driving the evolution and spread of phosphine resistance, as also suggested for *S. zeamais* (Fragoso *et al* 2003b). In contrast, the results found for *R. dominica* and *O. surinamensis* indicate that the capacity of dispersion of these insects can affect the dispersion of resistant genotypes, besides the local selection.

This assumption provides support for the hypothesis that the dispersion of insects of one place to another is relevant in the phosphine resistance evolution. The selection exercised by insecticides and the capacity of dispersion of a species are important information for the adoption of resistance management strategies (Georghiou 1972, Scott *et al* 2000). Besides the dispersion of the insects it is obvious that, particularly for stored-product insects, grain trade within the country is an important factor driving the spread of phosphine resistance. Regarding a more applied perspective, such studies will provide support for establishing the geographical scale of management required and the selection of suitable insecticides for use in rotation or in combination in insecticide resistance management programs.

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