

PS4-10 – 6243

Phosphine resistance, respiration rate and fitness consequences in *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

M.A.G. Pimentel^{1,*}, L.R.D'A. Faroni², R.N.C. Guedes¹, A.P. Neto², F.M. Garcia²

Abstract

Resistance to fumigants has been reported frequently in insect pests of stored products and is one of the main obstacles in controlling these pests. We studied phosphine resistance and the mechanism involved in 12 populations of *Tribolium castaneum* (Herbst) from Brazil and the possible existence of fitness costs associated with phosphine resistance in the absence of this fumigant. The phosphine resistance bioassays followed the standard method recommended by the Food and Agriculture Organization of the United Nations (FAO). The respiration rate (CO₂ production) and the instantaneous rate of increase (r_i) of each population were correlated to the resistance ratio (at LC₅₀). The resistance ratio in *T. castaneum* ranged from 1.0 to 186.2-fold and ten populations of *T. castaneum* showed phosphine resistance. The populations with lower respiration rate showed a higher resistance ratio and this are possibly related to the phosphine resistance mechanism. Conversely, populations with higher r_i showed lower resistance ratios. This suggests a lower rate of reproduction of the resistant populations compared with susceptible populations. Thus, management strategies that are based on the interruption of phosphine fumigation may result in reestablishment of the susceptibility.

Key words: Fumigation, insecticide resistance, red flour beetle, respiration rate, fitness cost.

Introduction

Phosphine has been a widely used fumigant for the control of stored-product insects for almost half century (Price and Mills, 1988; Chaudhry, 2000). It is by far the most widely used fumigant, because of its low cost, fast diffusion in air and absence of residues (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 resistance development in pest populations (Chaudhry and Price, 1990; Benhalima et al., 2004).

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 resistant was not found in Brazil at the time (Champ and Dyte, 1976). Since this survey many control failures with phosphine have been reported in Brazil, probably caused by resistance to this fumigant (Pacheco et al., 1990; Sartori et al., 1990). Except for the global survey undertaken by the FAO

¹ Department of Animal Biology, Federal University of Viçosa (UFV), Viçosa, MG, 36570-000, Brazil.

² Department of Agricultural Engineering, Federal University of Viçosa (UFV), Viçosa, MG, 36570-000, Brazil.

* Corresponding author. Department of Animal Biology, Universidade Federal de Viçosa, Viçosa, MG, 36570-000, Brazil. Phone: +55-31-3899-1919; Fax: +55-31-3899-2537. E-mail address: marco@insecta.ufv.br (MAG Pimentel).

(Champ and Dyte, 1976), surveys for phosphine resistance of stored-products insects in Brazil, are very few. Pacheco et al. (1990) and Sartori et al. (1990) showed that populations of *Rhyzopertha dominica* (F.), *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.) and *Cryptolestes ferrugineus* (Stephens) were highly resistant to phosphine. However, the levels of phosphine resistance in populations of *T. castaneum* from Brazil are not known because these studies were based on tests with discriminating concentration.

The survey and the monitoring of changes in the susceptibility of insects are fundamental for phosphine resistance management. They allow the assessment of the management strategies adopted to delay resistance evolution (Roush and Tabashnik, 1990). Phosphine resistance management studies are of particular interest in the control of resistant populations. Resistance to insecticides is often accompanied by fitness costs, such as a decreased rate of development, fecundity, survival, or mating competitiveness relative to susceptible insects (Roush and McKenzie, 1987). Differences in the biological parameters affecting the growth rate of insect populations are of particular interest to insecticide resistance management (Haubruge and Arnaud, 2001; Fragozo et al., 2005).

The mechanism of phosphine resistance of stored-product insects is also fundamental to the phosphine resistance management. One mechanism of phosphine resistance reported in these insects was the reduced uptake of the fumigant, a process designated as active exclusion (Price, 1981; 1984). The understanding of this mechanism of phosphine resistance can contribute to development new management strategies (Chaudhry, 1997).

Studies on mechanisms of resistance contribute to guide actions to be added to the integrated pest management programs. Management strategies also depend on detailed studies about detection and existence of adaptive costs in the absence of insecticides.

The aim of our study was (1) to assess the spread and severity of phosphine resistance in populations of *T. castaneum* in Brazil; (2) to verify if phosphine resistance in these populations

involves reduced respiration rate; and (3) to verify the existence of fitness cost associated with phosphine resistance by correlating the instantaneous rate of population growth (r_i), with the resistance levels.

Material and methods

Insects

We used twelve populations of *T. castaneum* collected at twelve places in four Brazilian states (Table 1). These populations were reared on broken maize grains (13 % m.c.) in glass jars (1.5 l) under controlled conditions (28 ± 2 °C, 70 ± 5 % r.h.).

Concentration-response curves

Fumigation of adults was based on the FAO method (FAO, 1975) and took place at 25 °C and 70 % r.h. Phosphine (of about 86 % purity) was produced using aluminium 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. There were 50 adults (males and females) per container and two containers per desiccator; 5 to 8 phosphine concentrations were used to establish each concentration-mortality curve. Phosphine was injected with a gas-tight syringes through a septum in the lid of each desiccator to produce the required concentration. After fumigation, the containers were removed from the desiccators and kept for 14 days (25 °C and 70 % r.h.), after which mortality was assessed.

Respirometry assays

The production of carbon dioxide (CO₂) was measured in a CO₂ analyser (TR 2 Sable System International, Las Vegas, USA) using methods adapted from Guedes et al. (2006). A series of 25-mL flasks was used, each flask containing 20 insects of each population in a completely closed

system. Three replicates were used for each population and CO₂ production was measured in each flask. CO₂-free air was injected into the flasks for two minutes at a 600 mL min⁻¹ flow.

Instantaneous rate of increase (r_i)

The instantaneous rate of increase (r_i) was measured in Petri dishes (140 x 10 mm) with 40 g of broken maize grains (13 % m.c.). Ten dishes were infested with 20 adults (males and females) of 2-4 weeks old, 25 ± 2 °C and 70 ± 5 % r.h. The number of alive and dead insects was counted after 60 days. The instantaneous rate of increase of each population was calculated as $r_i = \ln(N_f/N_0)/rt$, where, N_f is the final number of insects alive, N_0 is the initial number of insects and rt is the duration of the experiment (Walthall & Stark, 1997).

Statistical analysis

Concentration-response data was subjected to probit analysis (PROC PROBIT; SAS Institute, 1989), generating concentration-mortality curves. The resistance ratio (RR) and confidence intervals ($p < 0.95$) were calculated following Robertson and Preisler (1992). CO₂ production

(mmolCO₂ h⁻¹ insect⁻¹) and the instantaneous rate of increase (r_i) were subjected to analysis of variance followed by Tukey's multiple range tests, if appropriate ($p < 0.05$, PROC GLM; SAS Institute, 1989). The relationships between resistance levels and both r_i and respiration rate were subjected to regression analyses (SPSS, 2001).

Results

Phosphine resistance

Concentration-response curves for the populations of *T. castaneum* exposed to the phosphine are presented in Table 2. Mortality values predicted by the probit model did not differ significantly from the bioassay observed values ($p > 0.05$), and the probit model was therefore used for the concentration-mortality analyses. The concentration-response curves were used to identify a standard susceptible population, as well as resistant populations (Table 2). There was a large variation in susceptibility among populations. The concentration-mortality curves indicated that some populations of *T. castaneum* contained highly resistant individuals (Table 2).

Table 1. Origin, time and place of collection of Brazilian populations of *Tribolium castaneum*.

County	State	Facility	Time
Água Boa	Mato Grosso	Warehouse	August 2004
Aguanil	Minas Gerais	Farm wood store	May 2005
Alfenas	Minas Gerais	Metallic bin	April 2004
Bom Despacho	Minas Gerais	Metallic bin	May 2005
Bragança Paulista	São Paulo	Farm wood store	March 2005
Campos de Júlio	Mato Grosso	Metallic bin	June 2004
Nova Era	Minas Gerais	Warehouse	May 2005
Piracicaba	São Paulo	Laboratory	Agosto 2004
Rio Verde	Goiás	Metallic bin	July 2004
Uberlândia	Minas Gerais	Metallic bin	August 2004
Unaí	Minas Gerais	Metallic bin	August 2004
Viçosa	Minas Gerais	Laboratory	March 2004

Table 2. Toxicity of phosphine against twelve populations of *Tribolium castaneum*.

Population	N ¹	Slope ± SEM	LC ₅₀ (95% LL) mg l ⁻¹	RR ₅₀ (95% CL)	LC ₉₅ (95% FL) mg l ⁻¹	RR ₉₅ (95% CL)	X ²	p
Água Boa ²	650	3.46±0.39	0.02 (0.018-0.022)	1.0	0.06 (0.051-0.075)	1.0	8.99	0.11
Aguanil	500	2.37±0.22	0.02 (0.017-0.022)	1.0 (0.8-1.2)	0.10 (0.074-0.134)	1.6 (1.1-2.3)	0.94	0.82
Piracicaba	450	3.15±0.27	0.04 (0.036-0.049)	2.1 (1.7-2.5)	0.22 (0.168-0.315)	3.7 (2.6-5.3)	7.17	0.13
Bragança Paulista	400	0.81±0.13	0.05 (0.022-0.087)	2.6 (1.4-5.0)	5.64 (2.464-26.292)	95.6 (31.4-290.5)	4.43	0.22
Nova Era	400	7.92±0.65	0.07 (0.062-0.068)	3.2 (2.9-3.6)	0.10 (0.097-0.115)	1.7 (1.4-2.1)	1.23	0.54
Rio Verde	750	4.35±0.34	0.74 (0.677-0.794)	37.0 (32.4-42.2)	1.77 (1.613-1.986)	29.6 (24.0-36.5)	8.41	0.21
Viçosa	500	2.22±0.22	0.81 (0.717-0.920)	40.6 (34.5-47.8)	4.49 (3.333-6.922)	75.3 (50.3-112.9)	5.79	0.12
Uberlândia	800	3.39±0.36	0.84 (0.713-0.938)	41.9 (35.3-49.7)	2.56 (2.272-3.024)	42.8 (34.0-53.9)	7.31	0.29
Alfenas	650	2.67±0.24	0.96 (0.868-1.050)	47.9 (41.6-55.3)	3.95 (3.201-5.296)	66.2 (48.6-90.3)	4.72	0.19
Unaí	700	2.82±0.30	1.03 (0.838-1.188)	51.4 (42.1-62.9)	3.94 (3.406-4.854)	66.1 (51.3-85.1)	3.74	0.15
Campos de Júlio	850	2.39±0.18	1.25 (1.210-1.294)	62.7 (56.2-70.0)	2.09 (1.980-2.246)	35.0 (28.8-42.5)	2.28	0.68
Bom Despacho	650	3.91±0.26	3.72 (3.486-3.989)	186.2 (164.3-211.0)	9.80 (8.543-11.677)	164.1 (128.9-208.9)	3.99	0.14

¹N=Number of insects; ²Standard susceptible population.

Respiratory assays

The CO₂ production varied significantly among populations of *T. castaneum* (F=37.24, d.f.=102, $p < 0.0001$). The populations with the highest CO₂ production had the smallest resistance ratio (RR₅₀) (Figure 1). This confirms the hypothesis that smaller respiratory rates (CO₂ production) are correlated to high resistance levels. A reduction of respiration results in a reduced uptake of the fumigant.

Instantaneous rate of increase (r_i)

We used the instantaneous rate of increase (r_i) to assess the growth of resistant and susceptible populations. There was significant variation in r_i among populations of *T. castaneum* (F=12.21, d.f.=67, $p < 0.0001$). The populations with high r_i showed the smallest resistance ratio (Figure 2). This indicates the existence of adaptive costs of phosphine resistance.

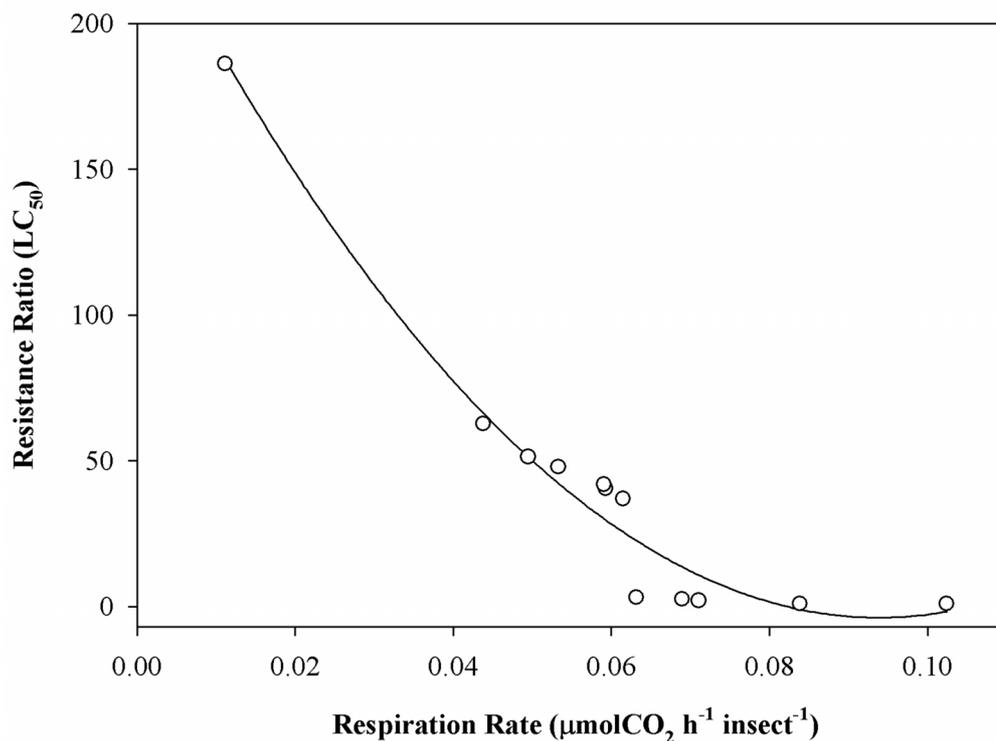


Figure 1. Phosphine resistance ratio as a function of respiration rate (CO₂ production) for populations of *Tribolium castaneum*. Symbols represent means of three replicates. ($y=242.7-5249.9x+27949.0x^2$; $r^2=0.97$; $F=125.03$; $p<0.0001$; d.f.=11).

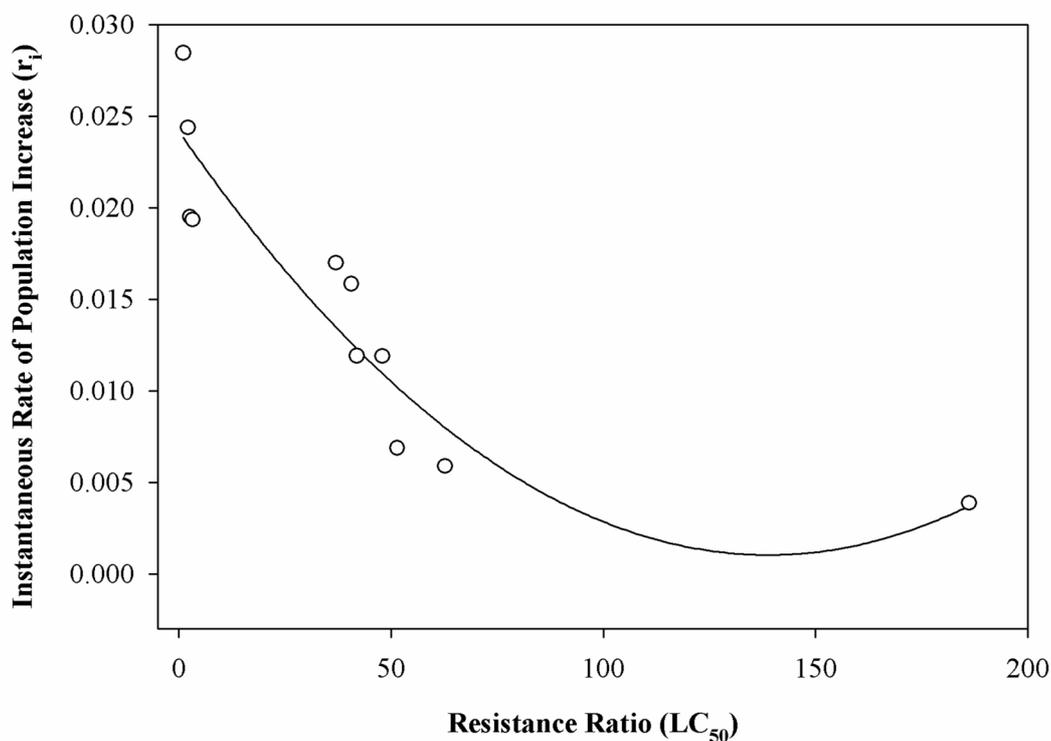


Figure 2. Instantaneous rate of increase (r_i) of populations of *Tribolium castaneum* as a function of phosphine resistance ratio. Symbols represent means of ten replicates. ($y=0.0241-0.0003x$; $r^2=0.85$; $F=22.82$; $p<0.0005$; d.f.=10).

Discussion

Our results indicate the occurrence of phosphine resistance in 10 populations of *T. castaneum* from the states of Goiás, Mato Grosso, Minas Gerais and São Paulo. This is the first report of phosphine resistance in stored product pests in the states of Mato Grosso and Minas Gerais. Hence, phosphine resistance is spread in Brazilian populations of the red flour beetle.

Our study show mid to high resistance levels (RR₅₀ between 10- and 100-fold). Some populations of *T. castaneum* showed high levels of phosphine resistance, with a LC₉₅ higher than the recommended dose of phosphine. These high resistance levels suggest that these populations have been under high selection pressure for many years (Benhalima et al., 2004). Fragoso et al. (2003) and Benhalima et al. (2004) suggested the movement of insects due to the trade of commodities as the probable factor for the spread of insecticide resistance.

The physiological mechanism of phosphine resistance is not completely elucidated. However, phosphine resistance has been associated with the active exclusion of phosphine by the gas exchange system (Price, 1981, 1984; Chaudhry and Price, 1990). This resistance mechanism is based on the excretion of poisonous compounds by the organism through the active exclusion of the gas through the respiratory system, associated with the reduction in the uptake of this gas (Price, 1984). The production of CO₂ reduces with the increase of the resistance reason for LC₅₀, indicating that the reduction of the respiration rate of the resistant strains leads to reduced uptake of the fumigant by the resistant insects.

The hypothesis that the mechanism of phosphine resistance in stored product pests is related to low uptake of the fumigant was tested in studies with the use of radioactive fumigants. These studies indicate that the mechanism of phosphine resistance is related to the reduction of the uptake of PH₃ (Chaudhry, 1997), which was already demonstrated in *R. dominica* (Price, 1981, 1984; Chaudhry and Price, 1990), *T. castaneum* (Nakakita and Kuroda, 1986),

Cryptolestes ferrugineus (Price and Dance, 1983) and *O. surinamensis* (Price and Dance, 1983). According to Benhalima et al. (2004), the exposure of some populations of *R. dominica*, *S. oryzae* and *T. castaneum* to [³²P]-radiolabelled phosphine showed that [³²P] uptake by resistant strains of all three species tested was much lower than that by susceptible strains. Under similar conditions of exposure (0.7 g m⁻³ of [³²P]-PH₃ for 5 hours to 25 °C), a susceptible strain of *T. castaneum* absorbed seven times more gas per g of insect than a resistant strain. Our data confirm findings by Benhalima et al. (2004), Price (1981, 1984), Chaudhry and Price (1990), Nakakita and Kuroda (1986) and Price and Dance (1983). Moreover, we show here that these results might be caused by the reduction of the respiratory rate of the resistant strains.

The process of reduction of the respiratory rate suggests that the main protecting mechanism against fumigants requires extra energy. According to Coustau et al. (2000), adaptations to unfavorable environments (resistance to insecticides) demand great changes in the phenotype resulting in an additional cost for the resistant strains. Indeed, the instantaneous rate of population growth (r_i) was negatively correlated with the resistance ratio (LC₅₀). The fact that the resistant populations have smaller r_i than the susceptible strains provides support for the hypothesis that the allocation of energy for the production of defense mechanisms against insecticides in the resistant strains impairs their reproductive performance in the absence of the fumigant.

Our data confirm findings by White and Bell (1990) and Longstaff (1991), who show that resistant strains of *S. oryzae* and *C. ferrugineus* had lower fecundity and intrinsic growth rates (r_m) than susceptible strains. Fragoso et al. (2005) also showed that an insecticide susceptible population of *S. zeamais* developed faster than a resistant strain. Moreover, we show here that these results might be caused by the allocation of energy for the production of defense mechanisms against insecticides in the resistant strains would be reducing the reproductive performance.

Resistant insects may have reduced fecundity, decrease survival of immature and mature stages, or prolonged developmental time of certain life stages when compared with susceptible insects. Populations of resistant insects would thus suffer disadvantages in the absence of insecticides, when the magnitude of the associated costs is negatively affect the performance or development of the population. Several studies on the evolution of resistance associate this phenomenon with an adaptative cost (White and Bell, 1990; Longstaff, 1991; Haubruge and Arnaud, 2001; Fragoso et al., 2005; Guedes et al., 2006). Regarding a more applied perspective, note that some resistant genotypes show fitness costs associated with insecticide resistance. In these cases, resistance management strategies that are based on the suppression of insecticide use aiming to re-establish insecticide susceptibility, and the eventual reintroduction of the insecticide can be used (Fragoso et al., 2005).

Acknowledgements

The authors would like to thank A.F. Rozado, V. Arthur, C.L. Cardoso, and D.P. Cardoso for providing *T. castaneum* populations used in this study; and the Companhia de Armazéns e Silos do Estado de Minas Gerais (CASEMG) and Companhia de Entrepostos e Armazéns Gerais de São Paulo (CEAGESP) for allowing insect sampling in their storage facilities. We thank Arne Janssen (Visiting Teacher, scholarship Professor Visitante Estrangeiro from CAPES, Brazil) for correcting the English. Financial support by CAPES (M.A.G.P.), CNPq and FAPEMIG was greatly appreciated.

References

Benhalima, H., Chaudhry, M.Q., Mills, K.A., Price, N.R., 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. *Journal of Stored Products Research* 40, 241-249.

Champ, B.R., Dyte, C.E., 1976. Report on the FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant Protection and Production Services No. 5, FAO, Rome.

Chaudhry, M.Q., Price, N.R., 1990. Insect mortality at doses of phosphine which produce equal uptake in susceptible and resistant strains of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). *Journal of Stored Products Research* 26, 101-107.

Chaudhry, M.Q., 1997. A review of the mechanisms involved in the action of phosphine as an insecticide and phosphine resistance in stored-product insects. *Pesticide Science* 49, 213-228.

Chaudhry, M. Q., 2000. Phosphine resistance. *Pesticide Outlook* 3, 88-91.

Coustau, C., Chevillon, C., ffrench-Constant, R. H., 2000. Resistance to xenobiotics and parasites: can we count the cost? *Trends in Ecology and Evolution* 15, 378-383.

FAO, 1975. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. 16: Tentative method for adults of some stored cereals, with methyl bromide and phosphine. FAO Plant Protection Bulletin 23, 12-25.

Fragoso, D.B., Guedes, R.N.C., Rezende, S.T., 2003. Glutathione S-transferase detoxification as a potential pyrethroid resistance mechanism in the maize weevil, *Sitophilus zeamais*. *Entomologia Experimentalis et Applicata* 109, 21-29.

Fragoso, D.B., Guedes, R.N.C., Peternelli, L.A., 2005. Developmental rates and population growth of insecticide-resistant and susceptible populations of *Sitophilus zeamais*. *Journal of Stored Product Research* 41, 271-281.

- Guedes, R.N.C., Oliveira, E.E., Guedes, N.M. P., Ribeiro, B., Serrão, J.E., 2006. Cost and mitigation of insecticide resistance in the maize weevil, *Sitophilus zeamais*. *Physiological Entomology* 31, 30-38.
- Haubruege, E., Arnaud, A., 2001. Fitness consequences malathion-specific resistance in red flour beetle (Coleoptera: Tenebrionidae) and selection for resistance in the absence of malathion. *Journal of Economic Entomology* 94, 552-557.
- Longstaff, B.C., 1991. An experimental study of the fitness of susceptible and resistant strains of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) exposed to insecticide. *Journal of Stored Products Research* 27, 75-82.
- Nakakita, H., Kuroda, J., 1986. Differences in phosphine uptake between susceptible and resistant strains of insects. *Journal of Pesticide Science* 11, 21-26.
- Pacheco, I.A., Sartori, M.R., Taylor, R.W.D., 1990. Levantamento de resistência de insetos-praga de grãos armazenados à fosfina, no estado de São Paulo. *Coletânea do ITAL* 20, 144-154.
- Price, N.R., 1981. A comparison of the uptake and metabolism of ³²P-radiolabelled phosphine in susceptible and resistant strains of the Lesser Grain Borer (*Rhyzopertha dominica*). *Comparative Biochemistry and Physiology* 69C, 129-131.
- Price, N.R., Dance, S.J., 1983. Some biochemical aspects of phosphine action and resistance in three species of stored product beetles. *Comparative Biochemistry and Physiology* 76, 277-281.
- Price, N.R., 1984. Active exclusion of phosphine as a mechanism of resistance in *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). *Journal of Stored Products Research* 20, 163-168.
- Price, L.A., Mills, K.A., 1988. The toxicity of phosphine to the immature stages of resistant and susceptible strains of some common stored product beetles, and implications for their control. *Journal of Stored Products Research* 24, 51-59.
- Robertson, J.L., Preisler, H.K., 1992. *Pesticide Bioassays with Arthropods*. CRC, Boca Raton, FL., USA.
- Roush, R.T., McKenzie, J.A., 1987. Ecological genetics of insecticide and acaricide resistance. *Annual Review of Entomology* 32, 361-380.
- Roush, R.T., Tabashnik, B.E., 1990. *Pesticide Resistance in Arthropods*. Chapman and Hall, London, UK.
- Sartori, M.R., Pacheco, I.A., Vilar, R.M., 1990. Resistance to phosphine in stored grain insects in Brazil. In: *Fifth International Working Conference on Stored-Product Protection*, v. 2, 1990, Bordeaux. *Proceedings...* Bordeaux. 1041-1050.
- SAS Institute. *SAS/STAT User's Guide*, version 6. 1989. SAS Institute, Cary, NC, USA. 1989.
- SPSS, 2001. *Sigma Plot User's Guide*, version 7.0. SPSS Inc., Chicago, USA.
- White, N.D.G., Bell, R.J., 1990. Relative fitness of a malathion-resistant strain of *Cryptolestes ferrugineus* (Coleoptera: Cucujidae) when development and oviposition occur in malathion-treated and untreated wheat kernels. *Journal of Stored Products Research* 26, 23-37.