CROP PROTECTION

The Cross-Resistance Spectrum in Deltamethrin Resistance Strains of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae)

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Resistência Cruzada em Raças de *Rhyzopertha dominica* (F.) (Coleoptera; Bostrychidae) Resistentes ao Inseticida Deltametrina

RESUMO - Objetivou-se verificar a existência de resistência cruzada em raças de *Rhyzopertha dominica* (F.) resistentes ao inseticida deltametrina. Os experimentos foram conduzidos testando outros três inseticidas, também usados para controlar pragas de grãos armazenados, como permetrina (piretróide), pirimifós-metil e clorpirifós-metil (organofosforados). Quatro raças da praga foram usadas: duas raças suscetíveis, BR4 e UK1, e duas raças resistentes, BR6 e BR7, todas mantidas em laboratório após a coleta nos armazéns. Inicialmente todas as raças foram avaliadas em sua susceptibilidade ao inseticida deltametrina. Posteriormente, cada raça foi submetida a pressão de seleção ao inseticida deltametrina por três gerações consecutivas e no final da terceira geração cada raça foi avaliada em sua suscetibilidade com todos os inseticidas para determinar a resistência cruzada. Os resultados mostraram a existência de resistência cruzada para o inseticida permetrina, nas raças BR6 e BR7 de *R. dominica*. Não houve resistência cruzada aos dois inseticidas organofosforados testados. Isso indica que a resistência cruzada ocorreu dentro do mesmo grupo químico de inseticidas e não entre diferentes grupos.

PALAVRAS-CHAVE: Insecta, pragas de grãos armazenados, permetrina, pirimifós-metil, clorpirifós-metil.

ABSTRACT - In order to verify if there was cross-resistance in deltamethrin resistance strains of *Rhyzopertha dominica* (F.), experiments were carried out with three insecticides used in stored grain to control pests: permethrin (pyrethroid), pirimiphos-methyl and chlorpyrifos-methyl (organophosphates). Three strains from Brazil (BR4, BR6 and BR7) and one from United Kingdom (UK1) were tested. The strains BR4 and UK1 were susceptible and BR6 and BR7 were resistant to deltamethrin. Initially all strains were assessed in their susceptibility to deltamethrin insecticide. Then, after they were submitted to deltamethrin selection pressure throughout three generations, each strain was assessed with all other insecticides to determine the cross-resistance spectrum. Results showed that cross-resistance to permethrin existed in the BR6 and BR7 strains of *R. dominica*. No cross-resistance to the organophosphates, pirimiphos-methyl and chlorpyrifos-methyl, was detected in deltamethrin-resistant strains.

KEY WORDS: Insecta, stored grain pest, permethrin, pirimiphos-methyl, chlorpyrifos-methyl.

The cross-resistance spectrum exhibited by a species is a very useful tool in detecting the mechanism involved in resistance of a pest to a specific pesticide (Scott 1990). The patterns of cross-resistance of the house fly *Musca domestica* L. showed that resistance to organophosphorus insecticides conferred resistance to carbamate but not to pyrethroids (Kudamatsu *et al*. 1983). This pattern was similar to one found by Champ and Campbell-Brown (Champ & Campbell-Brown 1970b), where malathion resistant strains of *Tribolium castaneum* (Herbst) were resistant also to several organophosphate insecticides but their tolerance of some pyrethroids was only slightly increased.

Strains of *Sitophilus zeamais* (Motsch.) exposed for a long time to DDT on grain developed resistance to DDT and the pyrethroids deltamethrin, cypermethrin and permethrin (Guedes *et al*. 1995). Also pyrethrin resistant strains of *Sitophilus granarius* (L.) showed resistance to DDT and other pyrethroids (Prickett 1980). This cross-resistance between DDT and pyrethroids was reported also in stored grain pests by Heather (Heather 1986), in which *Sitophilus oryzae* (L.) developed cross-resistance to pyrethroids after long term exposure to DDT.

Selecting for resistance to one pyrethroid in cotton bollworm, *Helicoverpa armigera* (Hubner), demonstrated cross-resistance to all other compounds in this group while no cross-resistance occurred to monocrotophos (Cheng Guilin & Yanchou 1995). This pattern also was reported by Cahill *et al*. (1995) in tobacco whitefly *Bemisia tabaci* (Genn.) where resistance to monocrotophos was not correlated with pyrethroid resistance but was with profenofos, showing different patterns of within the same chemical group. Deltamethrin resistance in cotton bollworm in the field rose from 94 to 56,910 fold during less than ten years and the same magnitude of resistance was found in the laboratory after 15 generations of selection with this insecticide (Cheng Guilin & Yanchou 1995).

Rhyzopertha dominica (F.) strain resistant to phoxim showed very high cross-resistance to chlorpyrifos with a resistance ratio of 468 but not to (Yao & Lo 1994), demonstrating no correlation in resistance between some organophosphorus and pyrethroid insecticides.

The aim of this work was to investigate the cross-resistance spectrum of deltamethrin resistant strains of *R. dominica* in order to help managing the resistance.

Material and Methods

Insecticides and Strains. Experiments were carried out with four insecticides used to control stored grain pests worldwide. The compounds were the pyrethroids, deltamethrin 27 g a.i./l (K-otec 25 EC) and permethrin 250 g i.a./l (Ambush 250 EC), and pirimiphos-methyl 500 g a.i./l (Actellic 500 EC) and chlorpyrifos-methyl 450 g a.i./l (Dursban 450 EC), both organophosphates. Deltamethrin was used as standard.

Three strains from Brazil (BR4, BR6 and BR7) and one from United Kingdom (UK1) were tested. The localities and year when collected from storage sites were: BR4 from Sertão-RS (1994), BR6 from Santa Rosa-RS (1993) and BR7 from Santo Cristo-RS (1994). Each strain had a different history of exposure to deltamethrin insecticide. The BR6 and BR7 storage sites had received repeated treatments of the compound for at least eight years before collection. The BR4 strain was collected from a storage site that had no recorded deltamethrin treatments and the UK1 strain had been in culture in the insectary at Silwood Park for many years and had also not been exposed to deltamethrin treatments. The strains BR4 and UK1 were susceptible and BR6 and BR7 were resistant to deltamethrin insecticide (Lorini & Galley 1999).

Bioassay Procedures. The bioassay procedures followed the recommended methods of the FAO (Anonymous 1969, 1974, Champ & Campbell-Brown 1970a) with some modifications required by this species. Each strain was bioassayed on filter paper with the use of five concentrations of each insecticide and a control. The insecticides were diluted with petroleum ether solvent to the required concentrations and 1.0 ml run onto 9.0 cm diameter filter paper, replicated four times.

The filter papers were left to dry before placing them in petri dishes. Ten 1 to 10 days old adults of *R. dominica* were tested into each replication. They were left in a incubator with temperature and humidity control at 27±1°C and 70±5% r.h., for 24h before assessment of the mortality.

Culture Method and Deltamethrin Selection. The strains were left in culture in the laboratory after collection from the field for some time before the first assessment of this research study. All the strains were cultured in glass jars sealed with arboseal and filter paper across their tops. Organically grown wheat grain, variety Axowa, was provided as food to maintain all the cultures which were kept at a temperature and humidity of 27±1°C and 70±5% r.h.

At the beginning of this study all strains were bioassayed and measured their susceptibility to each insecticide, then they were cultured at laboratory with deltamethrin pressure, each generation, for three generations. Deltamethrin selection was carried out for each strain by exposing for 24h the population to the appropriate LC_{50} on filter paper, previously determined. The survivors were cultured and selected again at the next generation. After the third generation of selection each strain was assessed with all other insecticides to determine the cross-resistance spectrum.

Data Assessment and Statistical Analysis. The evaluation was made by counting as responding insects, those which were unable to walk during a two-minute observation period. In order to obtain the LC_{50}s, confidence limits, intercept and slopes, the number of responding insects were analysed in GLIM version 3.77 (Crawley 1993) after correcting for control mortality (Abbott 1925), and the ratios between strains were calculated. The comparison between LC_{50}s was done by the GLIM Package using ANOVA and the "F" test from the sets of log-dose logit. Also, that criteria was used to determined the cross-resistance between strains.

Results and Discussion

The first assessment of deltamethrin resistant strains of *R. dominica* to the pyrethroid permethrin and the two organophosphate insecticides, pirimiphos-methyl and chlorpyrifos-methyl, showed the cross-resistance. Thus, the two deltamethrin most resistant strains, BR6 and BR7 (Lorini & Galley 1996, 1999) with resistance ratios of 69.0 and 174.8 times, showed resistance ratios of 8.1 and 24.2 times to permethrin, 4.7 and 19.8 times to pirimiphos-methyl, and 23.9 and 13.0 times to chlorpyrifos-methyl, respectively, compared with the BR4 strain. However, the deltamethrin susceptible strain, UK1, showed a resistance ratio of 11.5 to

pirimiphos-methyl, compared with BR4 (Table 1).

After selection of *R. dominica* strains with deltamethrin throughout three generations they showed cross-resistance not affected significantly BR7 strain. Also not affected significantly all strains tested with chlorpyrifos-methyl (Tables 1 and 2).

Table 1. Log-dose logit mortality parameters for adults of *R. dominica* strains, bioassayed with different insecticides, before deltamethrin selection. LC_{50} values in mg/cm^2 of each insecticide.

Strains	LC_{50}(95% conf. lim.)	a[1]	SE_a	b[2]	SE_b	Ratio[3]
Deltamethrin						
BR4	0.0117(0.0029-0.0316) A	1.495	0.2384	0.774	0.1341	1.0
UK1	0.0547(0.0272-0.1058) B	1.645	0.2750	1.303	0.1748	4.7
BR6	0.8079(0.2689-1.4900) C	0.135	0.2261	1.462	0.2943	69.0
BR7	2.0450(1.0530-3.3420) D	-0.474	0.2284	1.525	0.2653	174.8
Permethrin						
BR4	0.9228(0.5146-1.6390) A	0.058	0.2075	1.673	0.2182	1.0
UK1	1.2430(0.7106-2.1670) A	-0.169	0.2149	1.788	0.2338	1.3
BR6	7.4870(3.0490-22.050) B	-0.728	0.1790	0.833	0.1329	8.1
BR7	22.350(9.0680-75.630) B	-1.188	0.2046	0.880	0.1443	24.2
Pirimiphos-methyl						
BR4	0.2932(0.0603-0.7932) A	0.491	0.1974	0.921	0.1654	1.0
UK1	3.3710(1.5440-6.8030) C	-0.624	0.2203	1.183	0.1650	11.5
BR6	1.3860(0.6559-2.6390) B	-0.197	0.2142	1.391	0.2002	4.7
BR7	5.8010(3.3260-9.9840) C	-1.416	0.2942	1.854	0.2464	19.8
Chlorpyrifos-methyl						
BR4	0.4508(0.2118-0.9420) A	0.377	0.1778	1.088	0.0773	1.0
UK1	0.5149(0.2427-1.0750) A	0.314	0.1773	1.088	0.0773	1.1
BR6	10.770(5.1040-23.390) B	-1.123	0.1884	1.088	0.0773	23.9
BR7	5.8610(2.8030-12.490) B	-0.836	0.1828	1.088	0.0773	13.0

[1]LC_{50}s followed with the same letter are not significantly different ($P < 0.05$) within the same insecticide. F-test compared the sets of log-dose logit.
[2]Where slopes were not significantly different parallel lines have been fitted by GLIM version 3.77 (Royal Statistical Society (Crawley 1993)). Elsewhere best fit equations are shown.
[3]Ratio = Any LC_{50} divided by LC_{50} of BR4 strain, within each insecticide
a = intercept b = slope SE = Standard Error.

spectrum as described below.

Laboratory selection with deltamethrin significantly ($P<0.05$) enhanced the tolerance of deltamethrin in all strains with ratios varying from 16.4 in UK1 to 341.4 in BR4 (calculated from Tables 1 and 2), which demonstrated the selection pressure of deltamethrin insecticide well expressed by the heritability of resistance (Lorini & Galley 2000).

The deltamethrin selection slightly increased permethrin tolerance in BR4 and UK1 strains but significantly ($P<0.05$) increased the tolerance in BR6 and BR7 strains with resistance ratios of 12.7 and 14.1, respectively (calculated from Tables 1 and 2).

Deltamethrin selection significantly ($P<0.05$) decreased pirimiphos-methyl tolerance in BR4, UK1 and BR6 strains with resistance ratios of 0.7, 0.3 and 0.5, respectively, and

The cross-resistance of *R. dominica* deltamethrin resistance strains to permethrin was clear in BR6 and BR7 strains. Selecting the strains with deltamethrin increased the tolerance to permethrin (Tables 1 and 2). These strains had never been exposed to permethrin before because this insecticide was not used as a protectant in stored grain in Brazil since the strains were collected. These findings agree with Guedes *et al.* (1995) who found similar results with *Sitophilus zeamais* (Motsch.) in which the resistance to DDT conferred cross-resistance to several pyrethroids. Also in selecting strains for resistance to one pyrethroid, Cheng Guilin & Yanchou (1995) found resistance to several others demonstrating this clear cross-resistance pattern.

On the other hand, the strains selected with deltamethrin showed a decreased tolerance of the organophosphate

Table 2. Log-dose logit mortality parameters for adults of *R. dominica* from deltamethrin selected strains bioassayed with different insecticides. LC_{50} values in mg/cm^2 of each insecticide.

Strains	LC_{50}(95% conf. lim.)	a[1]	SE_a	b [**]	SE_b
Deltamethrin:					
BR4	3.9950(1.7120-12.990) B	-0.610	0.1854	1.014	0.1654
UK1	0.8963(0.4192-2.1630) A	0.050	0.1851	1.054	0.1534
BR6	67.460(33.820-243.10) C	-2.502	0.3623	1.368	0.2765
BR7	119.50(56.680-547.70) D	-3.243	0.4697	1.561	0.3318
Permethrin:					
BR4	1.4460(0.4933-4.2180) A	-0.107	0.1564	0.668	0.0644
UK1	2.3910(0.8242-7.0810) A	-0.252	0.1570	0.668	0.0644
BR6	95.480(28.750-390.50) B	-1.322	0.1787	0.668	0.0644
BR7	314.60(84.030-1551.0) B	-1.668	0.1923	0.668	0.0644
Pirimiphos-methyl:					
BR4	0.2049(0.0557-0.4749) A	1.131	0.2688	1.642	0.2449
UK1	1.1000(0.5351-1.8690) C	-0.068	0.2204	1.642	0.2449
BR6	0.7323(0.3168-1.3130) B	-0.222	0.2253	1.642	0.2449
BR7	2.9290(1.7320-4.7450) D	-0.766	0.2223	1.642	0.2449
Chlorpyrifos-methyl:					
BR4	0.5610(0.0447-1.1730) A	0.432	0.3068	1.720	0.5302
UK1	0.5265(0.0257-1.1660) A	0.444	0.3056	1.594	0.5154
BR6	7.8580(5.8340-11.700) C	-2.714	0.4596	3.031	0.5537
BR7	3.9300(2.7700-5.5750) B	-1.528	0.3407	2.570	0.4810

[1]LC_{50}s followed with the same letter are not significantly different ($P < 0.05$) within the same insecticide. F-test compared the sets of log-dose logit.
[2]Where slopes were not significantly different parallel lines have been fitted by GLIM version 3.77 (Royal Statistical Society (Crawley 1993)). Elsewhere best fit equations are shown.
a = intercept b = slope SE = Standard Error.

insecticide, pirimiphos-methyl, showing a negative selection or not significant changes in tolerance to the other organophosphate insecticide, chlorpyrifos-methyl, then no cross-resistance to this compounds was detected (Tables 1 and 2). Pirimiphos-methyl was highly effective against pyrethroid resistant strains of *S. zeamais* in Brazil and no organophosphate cross-resistance was found between strains (Guedes *et al.* 1995). Probably the genetical explanation of this type of cross-resistance may be that different genes are involved in the resistance pattern. Although, determining the cross-resistance spectrum is an important tool in investigating the mechanisms involved in resistance, this is limited to strains that do not exhibit multiple resistance otherwise it becomes very difficult to interpret the results in relation to the mechanisms (Scott 1990).

Deltamethrin selection demonstrated cross-resistance to another pyrethroid (permethrin) and no cross-resistance to organophosphate insecticides. Also the synergist piperonyl butoxide failed to completely suppress resistance in this species to deltamethrin (Lorini & Galley 2000), it is likely that the *kdr*-like insensitive nervous system mechanism is involved in resistance of *R. dominica* to deltamethrin, in which further studies are suggested to investigate.

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Literature Cited

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Anonymous. 1969. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides: general principles. FAO Pl. Prot. Bull. 17: 76-82.

Anonymous. 1974. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides: tentative method for adults of some major beetle pests of stored cereals with malathion or lindane -

FAO method Nº. 15. FAO Pl. Prot. Bull. 22: 127-137.

Cahill, M., F.J. Byrne, K. Gorman, I. Denholm & A.L. Devonshire. 1995. Pyrethroid and organophosphate resistance in the tobacco whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). Bull. Entomol. Res. 85: 181-187.

Champ, B.R. & M. Campbell-Brown. 1970 a. Insecticide resistance in Australian *Tribolium castaneum* (Herbst) - I. A test method for detecting insecticide resistance. J. Stored Prod. Res. 6: 53-70.

Champ, B.R. & M. Campbell-Brown. 1970 b. Insecticide resistance in Australian *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae) - II Malathion resistance in Eastern Australia. J. Stored Prod. Res. 6: 111-131.

Cheng Guilin, L.Y. & J. Yanchou. 1995. Cross resistant patterns of insecticide-selected strains of cotton bollworm [*Helicoverpa armigera* (Hübner)]. Res. Pest Manag. News 7: 13-15.

Crawley, M.J. 1993. Glim for ecologists. Oxford, Blackwell Scientific Publications, 379p.

Guedes, R.N.C., J.O.G. Lima, J.P. Santos & C.D. Cruz. 1995. Resistance to DDT and pyrethroids in Brazilian populations of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). J. Stored Prod. Res. 31: 145-150.

Heather, N.W. 1986. Sex-linked resistance to pyrethroids in *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). J. Stored Prod. Res. 22: 15-20.

Kudamatsu, A., A. Hayashi & R. Kano. 1983. Patterns of cross resistance to insecticides in the house fly in Japan, p.411-419. In G.P. Georghiou & T. Saito (ed.), Pest resistance to pesticides: challenges and prospects. New York, Plenum Press, 797p.

Lorini, I. & D.J. Galley. 1996. Changes in resistance status of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), a pest of stored grain in Brazil, with and without deltamethrin selection. Res. Pest Manag. News 8: 12-14.

Lorini, I. & D.J. Galley. 1999. Deltamethrin resistance in *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), a pest of stored grains in Brazil. J. Stored Prod. Res. 35: 37-45.

Lorini, I. & D.J. Galley. 2000. Estimation of realized heritability of resistance to deltamethrin insecticide in selected strains of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). J. Stored Prod. Res. 36: 119-124.

Prickett, A.J. 1980. The cross-resistance spectrum of *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) heterozygous for pyrethrin resistance. J. Stored Prod. Res. 16: 19-25.

Scott, J.G. 1990. Investigating mechanisms of insecticide resistance: methods, strategies, and pitfalls, p.39-57. In R.T. Roush & B.E. Tabashnik (ed.), Pesticide resistance in arthropods. London, Chapman and Hall, 303p.

Yao, M.C. & K.C. Lo. 1994. Phoxin resistance in *Rhyzopertha dominica* Fabricius in Taiwan. Chinese J. Entomol. 14: 331-341.

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