CROP PROTECTION

Effect of the Synergists Piperonyl Butoxide and DEF in Deltamethrin Resistance on Strains of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae)

IRINEU LORINI[1] AND DAVID J. GALLEY[2]

[1]Empresa Brasileira de Pesquisa Agropecuária - Embrapa Trigo, Rodovia BR 285, km 174, Caixa postal 451, 99001-970, Passo Fundo, RS, Brazil. E-mail: ilorini@cnpt.embrapa.br

[2]Department of Biology. Imperial College of Science, Technology and Medicine. University of London. Silwood Park, Ascot, Berkshire, United Kingdom. SL5 7PY.

An. Soc. Entomol. Brasil 29(4): 749-755 (2000)

Efeito dos Sinergistas Butóxido de Piperonila e DEF em Raças de *Rhyzopertha dominica* (F.) Resistentes à Deltametrina

RESUMO - Os sinergistas são uma importante ferramenta de laboratório para auxiliar na determinação dos mecanismos de resistência de uma população de insetos. Esse tipo de investigação tem gerado resultados de grande valor no entendimento dos mecanismos de resistências aos pesticidas. Neste trabalho, foi testado o efeito de sinergistas na supressão da resistência de *Rhyzopertha dominica* (F.) resistentes ao inseticida deltametrina. Quatro raças da praga foram avaliadas: duas raças susceptíveis, BR4 e UK1, mantidas em laboratório por oito gerações consecutivas sem qualquer exposição à deltametrina; e duas raças resistentes, BR6 e BR7, mantidas em constante seleção por cinco gerações em laboratório com o inseticida. Cada raça foi avaliada em bioensaios com deltametrina impregnada em papel filtro, combinado com quatro proporções de cada sinergista (butóxido de piperonila e DEF), separadamente. As proporções foram: 1:0, 1:5, 1:10 e 1:15, de deltametrina e do sinergista, respectivamente. Embora em diferentes proporções, ambos sinergistas bloquearam as enzimas envolvidas na resistência da praga. O butóxido de piperonila conferiu os maiores índices de toxicidade sobre as raças resistentes, reduzindo em até 27 vezes o índice de rsistência do inseto, enquanto que DEF mostrou pouco efeito, reduzindo em no máximo cinco vezes a resistência das mesmas raças do inseto. Mesmo assim, a resistência não foi totalmente suprimida, sugerindo que outros mecanismos do inseto estão associados à resistência de deltametrina, além do metabolismo pelas enzimas oxidases e esterases.

PALAVRAS-CHAVE: Insecta, grãos armazenados, resistência a inseticidas.

ABSTRACT - Synergists are an important research tool in the laboratory to help to determine the mechanisms of resistance involved in a particular population. This kind of investigation has produced valuable results in understanding resistance to pesticides. The ability of these compounds in

suppressing deltamethrin resistance on four strains of *Rhyzopertha dominica* (F.) was evaluated under laboratory conditions. Two susceptible strains, obtained from the eighth generation in laboratory without any selection were tested; other two resistant strains consisted in the fifth deltamethrin selected generation. Each strain was bioassayed on filter paper impregnated with deltamethrin associated with a synergist in different proportions, following the methods recommended by FAO for assessing resistance. The synergists tested were piperonyl butoxide (PBO) and S,S,S-tributyl phosphorotrithioate (DEF), at the following proportions 1:0, 1:5, 1:10 and 1:15 of deltamethrin and the synergist, respectively. Both synergists showed a role in blocking enzymes involved in detoxifying deltamethrin. PBO conferred up to 27 times higher toxicity ratios in the resistant strains than in the susceptible ones. DEF was less effective in reducing the lethal dose of deltamethrin with ratios up to five times.

KEY WORDS: Insecta, stored grain, insecticide resistance, resistance mechanisms.

Resistance of pests to pesticides is an example of evolution of the species showing how they can survive and change physiologically under pressure of chemicals which select for genetical traits. Synergists are an important research tool in the laboratory to help in determining the mechanism of resistance involved in a particular population (Raffa & Priester 1985). This kind of investigation has produced valuable results in understanding resistance to pesticides. Some suppression of resistance may be achieved by adding synergists to the insecticide (Brindley & Selim 1984). These compounds block the mechanism of resistance and since metabolism is the main mechanism involved in resistance, the pest succumbs to the insecticide. However they have had little practical success in the field because the pests may also develop resistance to the synergists together with the insecticide.

Synergists have been used largely to overcome resistance and help to control various species of pests in the field and particularly in stored grain environments. Piperonyl butoxide has been used as a synergist with organophosphate and pyrethroid insecticides to control stored grain pests with excellent results as the 1:10 proportion between deltamethrin and piperonyl butoxide used in the market (Ardley 1976, Bengston *et al.* 1983, Samson *et al.* 1990, Daglish *et al.* 1995). Synergists can also preserve beneficial insects when mixed with insecticides and in two instances at least have shown less synergism against the beneficial insects than the pests (Plapp 1979, Rajakulendran & Plapp 1982).

When investigating mechanisms of insecticide resistance of a pest, several approaches may be employed. Although not all these techniques provide irrefutable evidence by themselves, the use of multiple methods may permit a final identification. Usually the best strategy in order to identify mechanisms of resistance is to begin with comparisons of synergism and cross-resistance data. From this information it is usually possible to formulate some idea about what the mechanism may be (Scott 1990).

Following this line of enquiry, the investigation of mechanisms of resistance in the *Rhyzopertha dominica* (F.) strains, resistant to deltamethrin, was started by measuring the effect of two synergists.

Material and Methods

Strains, Synergists and Treatments. Two susceptible (BR4 and UK1) and two resistant

(BR6 and BR7) strains of *R. dominica* (F.) to deltamethrin (Lorini & Galley 1996, 1999) were used to carry out these experiments. Adults of BR4 and UK1 strains were obtained from eighth laboratory generation without any selection and BR6 and BR7 strains from the fifth deltamethrin selected generation. Each strain was bioassayed on filter paper impregnated with deltamethrin associated with a synergist in different proportions, following the methods recommended by FAO for assessing resistance. The synergists tested were piperonyl butoxide (PBO) and S,S,S-tributyl phosphorotrithioate (DEF), at the following proportions 1:0, 1:5, 1:10 and 1:15 of deltamethrin and the synergist, respectively.

Bioassay Procedure. The bioassay procedures followed were the methods recommended by FAO (Anonymous 1974) with some modifications required by this species. Each strain was bioassayed on filter paper impregnated with the concentrations of deltamethrin plus the synergist. A control, without insecticide, was also used. The required concentrations of deltamethrin plus the synergists were diluted with petroleum ether solvent and 1.0 ml of this solution was run onto a 9.0 cm in diameter filter paper inside a 2 cm petri dish, with four replications. The filter papers were left to dry before placing the insects in petri dishes. Ten 1-10 day old adults of *R. dominica*, were released into each replication. The petri dishes were than transferred to an incubator at 27±1ºC and 70±5% R.H.

Data Assessment and Statistical Analysis. Those insects unable to walk during a two minutes observation period were counted. In order to obtain the LC_{50}s, confidence limits, intercept and slopes, the number of responding insects were analyzed by GLIM version 3.77 (Royal Statistical Society (Crawley 1993)) after correcting for control mortality (Abbott 1925), and the ratios of insect tolerance between deltamethrin and different proportions of the synergist 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.

Results and Discussion

Piperonyl Butoxide (PBO). This synergist enhanced the toxicity of deltamethrin in a dose-dependent manner in all proportions in the resistant strains. However, for the susceptible strains, the addition of PBO significantly ($P < 0.05$) enhanced the toxicity only in the higher proportions tested. (Table 1). Piperonyl butoxide conferred higher toxicity ratios in the resistant strains than in the susceptible ones. In this connection the biggest ratio between the LC_{50} without PBO divided by the LC_{50} with PBO on BR7 strain was 27 times with the 1: 15 combination while it was only four times in the susceptible BR4 strain at the same combination (Table 1).

S,S,S-Tributyl Phosphorotrithioate (DEF). Although this synergist reduced the tolerance of the BR7 strain up to five times, no significant differences ($P > 0.05$) in the LC_{50}s between any combination of insecticide and DEF were found in BR4, UK1, BR6 and BR7 strains. The ratios between the LC_{50} without DEF divided by the LC_{50} with the synergist were very low within strains showing a poor synergist effect (Table 2).

The additionn of the synergist PBO to deltamethrin effectively reduced the median lethal dose of the *R. dominica* strains tested and the 1:15 proportion between deltamethrin and this synergist reduced the ratio between susceptible (BR4) and resistant strains (BR7) from 4663 to 763 times. But because the resistance was not completely suppressed it is possible to infer that another mechanism of resistance besides the metabolism by oxidases may also be involved with these strains.

As PBO synergized deltamethrin in these experiments and since it is known to block mixed function oxidases (MFO) enzymes (Casida 1970, Ishaaya *et al.* 1987, Dittrich *et al.* 1990), it is possible to infer that the MFO enzymes play an important role in

Table 1. Log-dose logit mortality parameters for adults of *R. dominica* strains BR4, UK1, BR6 and BR7, bioassayed with deltamethrin (Del) + different proportion of piperonyl butoxide (PBO). LC_{50} values in mg/cm^2 of deltamethrin.

Proportion Del + PBO	LC_{50} (95% conf. lim.)[1]	a	SE_a	b	SE_b	Ratio[2]
BR4:						
01+00	0.0288(0.0099-0.0710) B	1.539	0.2159	0.999	0.1112	-
01+05	0.0166(0.0052-0.0430) AB	1.779	0.2264	0.999	0.1112	1.7
01+10	0.0082(0.0022-0.0229) AB	2.687	0.2420	0.999	0.1112	3.5
01+15	0.0065(0.0017-0.0189) A	2.182	0.2474	0.999	0.1112	4.4
UK1:						
01+00	0.0710(0.0304-0.1530) B	1.303	0.2103	1.135	0.1129	-
01+05	0.0296(0.0116-0.0672) AB	1.734	0.2266	1.135	0.1129	2.4
01+10	0.0161(0.0058-0.0383) A	2.036	0.2402	1.135	0.1129	4.4
01+15	0.0110(0.0037-0.0272) A	2.223	0.2496	1.135	0.1129	6.4
BR6:						
01+00	48.490(23.080-258.50) C	-1.890	0.3950	1.121	0.3033	-
01+05	4.7520(0.00001-19.76) B	-0.379	0.3183	0.559	0.2677	10.2
01+10	4.2230(0.8802-9.1360) A	-0.314	0.3271	0.981	0.2863	11.5
01+15	2.5580(0.0002-8.5670) A	-0.251	0.3189	0.615	0.2727	18.9
BR7:						
01+00	134.30(51.840-537.10) D	-1.981	0.2492	0.931	0.1416	-
01+05	17.750(7.9090-43.200) C	-1.163	0.2258	0.931	0.1416	7.6
01+10	9.0980(3.8690-20.670) B	-0.893	0.2214	0.931	0.1416	14.8
01+15	4.9630(1.9300-11.110) A	-0.648	0.2191	0.931	0.1416	27.1

[1]LC_{50}s followed by the same letter are not significantly different ($P > 0.05$) within the same strain. F-test compared the sets of log-dose logit.
[2]Ratio = LC_{50} without PBO divided by LC_{50} with PBO, within each strain
a = intercept b = slope SE = Standard Error

deltamethrin resistance of the *R. dominica* strains evaluated in this study. Since DEF synergist is involved in hydrolytic activity blocking the esterase enzymes (Jao & Casida 1974) and these experiments showed no significant effect of this synergist in *R. dominica*, it is possible to infer that esterase enzymes may not be involved in deltamethrin resistance of *R. dominica*.

Although the metabolic mechanism, as discussed above, plays an important role in resistance as demonstrated by the enzyme blockers PBO, reducing the tolerance of the strains, they failed to completely suppress resistance in this species, suggesting the involvement of other mechanisms.

Further studies are suggested to investigate the resistance in the nervous

Table 2. Log-dose logit mortality parameters for adults of *R. dominica* strains BR4, UK, BR6 and BR7, bioassayed with deltamethrin (Del) + different proportion of S,S,S-tributyl phosphorotrithioate (DEF). LC_{50} values in mg/cm^2 of deltamethrin.

Proportion Del + DEF	LC_{50}(95% conf. lim.)[1]	a	SE_a	b	SE_b	Ratio[2]
BR4:						
01+00	0.0376(0.0247-0.1243) A	1.435	0.2149	1.158	0.1114	-
01+05	0.0305(0.0123-0.0681) A	1.755	0.2271	1.158	0.1114	1.2
01+10	0.0217(0.0084-0.0498) A	1.925	0.2345	1.158	0.1114	1.7
01+15	0.0237(0.0093-0.0539) A	1.882	0.2326	1.158	0.1114	1.6
UK1:						
01+00	0.0691(0.0260-0.1666) A	1.107	0.1958	0.954	0.0965	-
01+05	0.0201(0.0065-0.0525) A	1.619	0.2138	0.954	0.0965	3.4
01+10	0.0415(0.0148-0.1028) A	1.319	0.2024	0.954	0.0965	1.6
01+15	0.0494(0.0180-0.1209) A	1.247	0.2001	0.954	0.0965	1.4
BR6:						
01+00	40.910(14.910-143.20) A	-1.774	0.2381	1.100	0.1186	-
01+05	19.740(7.8940-59.960) A	-1.425	0.2202	1.100	0.1186	2.0
01+10	13.560(5.6350-38.720) A	-1.246	0.2123	1.100	0.1186	3.0
01+15	10.380(4.4160-28.490) A	-1.118	0.2073	1.100	0.1186	3.9
BR7:						
01+00	118.70(28.050-905.30) A	-1.653	0.2268	0.798	0.1056	-
01+05	59.960(15.970-373.70) A	-1.417	0.2128	0.798	0.1056	1.9
01+10	46.600(12.920-270.90) A	-1.329	0.2082	0.798	0.1056	2.5
01+15	20.670(6.4170-97.850) A	-1.048	0.1956	0.798	0.1056	5.7

[1]LC_{50}s followed with the same letter are not significantly different ($P > 0.05$) within the same strain. F-test compared the sets of log-dose logit.
[2]Ratio = LC_{50} without DEF divided by LC_{50} with DEF, within each strain.
a = intercept b = slope SE = Standard Error.

system with emphasis on the gene involved in *kdr*-like knockdown resistance mechanism related to pyrethroids (Chang & Plapp 1983, Miller *et al.* 1983, Soderlund & Bloomquist 1990) which is the most important nervous system mechanism of resistance and seems to be involved in the *R. dominica* resistant strains.

Acknowledgement

The first author gratefully acknowledges the support of the Brazilian Agricultural Research Corporation - Embrapa for this

work.

Literature Cited

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

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.

Ardley, J.H. 1976. Synergized bioresmethrin as a potential grain protectant. J. Stored Prod. Res. 12: 253-259.

Bengston, M. , R.A.H. Davies, J.M. Desmarchelier, R. Henning, W. Murray, B.W. Simpson, J.T. Snelson, R. Sticka & B.E. Wallbank. 1983. Organophosphorothioates and synergised synthetic pyrethroids as grain protectants on bulk wheat. Pestic. Sci. 14: 373-384.

Brindley, W.A. & A.A. Selim. 1984. Synergism and antagonism in the analysis of insecticide resistance. Environ. Entomol. 13: 348-353.

Casida, J.E. 1970. Mixed-function oxidase involvement in the biochemistry. J. Agric. Food Chem. 18: 753-772.

Chang, C.P. & F.W.J. Plapp. 1983. DDT and pyrethroids: receptor binding in relation to knockdown resistance (kdr) in the house fly. Pestic. Biochem. Physiol. 20: 86-91.

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

Daglish, G.J. , M. Eelkema & L.M. Harrison. 1995. Chlorpyrifos-methyl plus either methoprene or synergised phenothrin for control of Coleoptera in maize in Queensland, Australia. J. Stored Prod. Res. 31: 235-241.

Dittrich, V., G.H. Ernst, O. Ruesh & S. Uk. 1990. Resistance mechanisms in sweetpotato whitefly (Homoptera: Aleyrodidae) populations from Sudan, Turkey, Guatemala, and Nicaragua. J. Econ. Entomol. 83: 1665-1670.

Gunning, R.V., G.D. Moores & A.L. Devonshire. 1996. Esterases and esfenvalerate resistance in Australian *Helicoverpa armigera* (Hubner) Lepidoptera: Noctuidae. Pestic. Biochem. Physiol. 54: 12-23.

Ishaaya, I., Z. Mendelson, K.R. Simon Ascher & J.E. Casida. 1987. Cypermethrin synergism by pyrethroid esterase inhibitors in adults of the whitefly *Bemisia tabaci*. Pestic. Biochem. Physiol. 28: 155-162.

Jao, L.T. & J.E. Casida. 1974. Esterases inhibitors as synergists for (+)-trans-chrysanthemate insecticide chemicals. Pestic. Biochem. Physiol. 4: 456-464.

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.

Miller, T.A. , V.L. Salgado & S.N. Irving. 1983. The *KDR* factor in pyrethroid resistance, 353-366. In G.P Georghiou & T. Saito (ed.), Pest resistance to

pesticides: challenges and prospects. New York, Plenum Press, 797p.

Plapp, W.J. 1979. Synergism of pyrethroid insecticides by formamidines against *Heliothis* pests of cotton. J. Econ. Entomol. 72: 667-670.

Raffa, K.F. & T.M. Priester. 1985. Synergists as research tools and control agents in agriculture. J. Agric. Entomol. 2: 27-140.

Rajakulendran, S.V. & F.W.J. Plapp. 1982. Synergism of five synthetic pyrethroids by chlordimeform against the tobacco budworm (Lepidoptera: Noctuidae) and a predator, *Chrysopa carnea* (Neuroptera: Chrysopidae). J. Econ. Entomol. 75: 1089-1092.

Samson, P.R. , R.J. Parker & E.A. Hall. 1990. Synergised deltamethrin as a protectant against *Sitophilus zeamais* Motsch. and *S. oryzae* (L.) (Coleoptera: Curculionidae) on stored maize. J. Stored Prod. Res. 26: 155-161.

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

Soderlund, D.M. & J.R. Bloomquist. 1990. Molecular mechanisms of insecticide resistance, 58 - 96. In R.T. Roush & B. E. Tabashnik (ed.), Pesticide resistance in arthropods. London, Chapman and Hall, 303p.

Accepted 10/IX/2000.