CROP PROTECTION

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

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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 a resistência 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 then transferred to an incubator at 27±1°C 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 addition 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...
The 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 system.

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/cm² of deltamethrin.

<table>
<thead>
<tr>
<th>Proportion Del + PBO</th>
<th>LC_{50} (95% conf. lim.)</th>
<th>a</th>
<th>SE_a</th>
<th>b</th>
<th>SE_b</th>
<th>Ratio²</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR4: 01+00</td>
<td>0.0288 (0.0099-0.0710) B</td>
<td>1.539</td>
<td>0.2159</td>
<td>0.999</td>
<td>0.1112</td>
<td>-</td>
</tr>
<tr>
<td>01+05</td>
<td>0.0166 (0.0052-0.0430) AB</td>
<td>1.779</td>
<td>0.2264</td>
<td>0.999</td>
<td>0.1112</td>
<td>1.7</td>
</tr>
<tr>
<td>01+10</td>
<td>0.0082 (0.0022-0.0229) AB</td>
<td>2.687</td>
<td>0.2420</td>
<td>0.999</td>
<td>0.1112</td>
<td>3.5</td>
</tr>
<tr>
<td>01+15</td>
<td>0.0065 (0.0017-0.0189) A</td>
<td>2.182</td>
<td>0.2474</td>
<td>0.999</td>
<td>0.1112</td>
<td>4.4</td>
</tr>
<tr>
<td>UK1: 01+00</td>
<td>0.0710 (0.0304-0.1530) B</td>
<td>1.303</td>
<td>0.2103</td>
<td>1.135</td>
<td>0.1129</td>
<td>-</td>
</tr>
<tr>
<td>01+05</td>
<td>0.0296 (0.0116-0.0672) AB</td>
<td>1.734</td>
<td>0.2266</td>
<td>1.135</td>
<td>0.1129</td>
<td>2.4</td>
</tr>
<tr>
<td>01+10</td>
<td>0.0161 (0.0058-0.0383) A</td>
<td>2.036</td>
<td>0.2402</td>
<td>1.135</td>
<td>0.1129</td>
<td>4.4</td>
</tr>
<tr>
<td>01+15</td>
<td>0.0110 (0.0037-0.0272) A</td>
<td>2.223</td>
<td>0.2496</td>
<td>1.135</td>
<td>0.1129</td>
<td>6.4</td>
</tr>
<tr>
<td>BR6: 01+00</td>
<td>48.490 (23.080-258.50) C</td>
<td>-1.890</td>
<td>0.3950</td>
<td>1.121</td>
<td>0.3033</td>
<td>-</td>
</tr>
<tr>
<td>01+05</td>
<td>4.7520 (0.00001-19.76) B</td>
<td>-0.379</td>
<td>0.3183</td>
<td>0.559</td>
<td>0.2677</td>
<td>10.2</td>
</tr>
<tr>
<td>01+10</td>
<td>4.2230 (0.8802-9.1360) A</td>
<td>-0.314</td>
<td>0.3271</td>
<td>0.981</td>
<td>0.2863</td>
<td>11.5</td>
</tr>
<tr>
<td>01+15</td>
<td>2.5580 (0.0002-8.5670) A</td>
<td>-0.251</td>
<td>0.3189</td>
<td>0.615</td>
<td>0.2727</td>
<td>18.9</td>
</tr>
<tr>
<td>BR7: 01+00</td>
<td>134.30 (51.840-537.10) D</td>
<td>-1.981</td>
<td>0.2492</td>
<td>0.931</td>
<td>0.1416</td>
<td>-</td>
</tr>
<tr>
<td>01+05</td>
<td>17.750 (7.9090-43.200) C</td>
<td>-1.163</td>
<td>0.2258</td>
<td>0.931</td>
<td>0.1416</td>
<td>7.6</td>
</tr>
<tr>
<td>01+10</td>
<td>9.0980 (3.8690-20.670) B</td>
<td>-0.893</td>
<td>0.2214</td>
<td>0.931</td>
<td>0.1416</td>
<td>14.8</td>
</tr>
<tr>
<td>01+15</td>
<td>4.9630 (1.9300-11.110) A</td>
<td>-0.648</td>
<td>0.2191</td>
<td>0.931</td>
<td>0.1416</td>
<td>27.1</td>
</tr>
</tbody>
</table>

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

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\begin{table}
\centering
\caption{Log-dose logit mortality parameters for adults of \textit{R. dominica} strains BR4, UK, BR6 and BR7, bioassayed with deltamethrin (Del) + different proportion of S,S,S-tributyl phosphorotrithioate (DEF). \textit{LC}_{50} values in mg/cm$^2$ of deltamethrin.}
\begin{tabular}{llllllll}
\hline
Proportion & Del + DEF & \textit{LC}_{50} (95\% conf. lim.)$^1$ & a & \textit{SE}_a & b & \textit{SE}_b & Ratio$^2$
\hline
\textbf{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
\textbf{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
\textbf{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
\textbf{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
\hline
\end{tabular}
\end{table}

$^1$\textit{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 = \textit{LC}_{50} without DEF divided by \textit{LC}_{50} with DEF, within each strain.
a = intercept  b = slope  \textit{SE} = Standard Error.
Literature Cited


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