RESISTANCE TO MALATHION, PIRIMIPHOS-METHYL AND FENITROTHION IN COLEOPTERA FROM STORED GRAINS.

Ivânia A. PACHECO; M. Regina SARTORI; Scheilla BOLONHEZI
Instituto de Tecnologia de Alimentos
Avenida Brasil, 2880 - Caixa Postal 139
Campinas - São Paulo - Brasil - CEP 13073

SUMMARY

The objectives of this paper were:1) to verify the occurrance of resistance to malathion, pirimiphos-methyl and fenitrothion in populations of coleoptera from stored grains and 2) to obtain data that could contribute to the adequate control of these insects during storage, thus reducing losses.

Populations of Sitophilus oryzae, S. zeamais, Tribolium castaneum and Rhyzopertha dominica were collected from storage facilities located in different regions of Brazil, and tested for resistance to the tree insecticides according to the FAO Standard Method (FAO, 1975). Insects were collected in the States of São Paulo, Rio Grande do Sul, Santa Catarina, Goiás, Acre and Rondonia. Twenty populations of S. oryzae, ten of S. zeamais, twenty of R. dominica and twenty-five of T. castaneum had already been tested for resistance to the three insecticides.

Seven populations of S. oryzae, six of R. dominica and ten of S. zeamais were susceptible to the three insecticides.

Resistance to malathion was showed in thirteen populations of S. oryzae, fourteen of R. dominica and twenty-five of T. castaneum.

Simultaneous cross-resistance to pirimiphos-methyl and fenitrothion was indicated in one population of S. oryzae and eight of T. castaneum.

Cross resistance only to pirimiphos-methyl was indicated in two populations of S. oryzae, one of R. dominica and one of T. castaneum and only to fenitrothion in two of R. dominica and two of T. castaneum.

INTRODUCTION

The continual usage of pesticides, mainly when used in an inadequate way, has lead to the development of insect resistance, this problem having intensified in recent years. Prior to 1940, only eight insect species were known to have developed resistance. By 1969 there were already reports of 224 insect species which had developed resistance to various insecticides (BROWN, 1961 and 1969, related by BANSODE, 1979).

A FAO Global survey carried out during 1972/73 (CHAMP & DYTE, 1976) showed insect resistance to malathion in 78 of the 86 countries in which samples were collected. In this survey resistance was detected in brazilian populations of Tribolium castaneum Herbst, 1797, T. confusum Du Val, 1868, Oryzaephilus surinamensis (L., 1758) and Rhyzopertha dominica (Fabr., 1792). Resistance was not detected in twenty three brazilian populations of Sitophilus oryzae (L.,1763) and S. zeamais (Mots., 1855).

Resistance to malathion in grain-storage-insects was also related by other authors in several parts of the world, such as in the United States (ZETTLER, 1974a e 1982; ZETTLER et alii,1973; HALISCAK & BEEMAN,1983; HORTON,1984; COGAN, 1982), India (SRIVASTAVA, 1980), Kenya, Nigeria, Argentina and Republic of

S. Africa (COGAN, 1982), Uganda (EVANS, 1985), Nepal (BINNS) and Australia (ATTIA, 1977; CHAMP & CAMPBELL BROWN, 1970; ATTIA & FRECKER, 1984).

The resistance mechanisms developed by the insects can be of different types and one population can exhibit more than one mechanism. One of the known mechanisms of resistance involves a change in the target site of the insecticide, which is acethylcholinesterase in the case of organophosphate and carbamate insecticides. (PLAPP Jr., 1986). According to ATTIA (1981), the most important mechanism of resistance to organophosphorus compounds in insects is to enhance detoxification by a number of enzymes such as carboxyesterases, phosphorotriester hydrolases, glutathion S-transferases and mixed function oxidases.

Increased carboxyesterase activity explains most of the cases of malathion resistance in strains of stored-product insects (DYTE & ROWLAND, 1968; CHAMP & DYTE, 1976). This mechanism, known as specific resistance, is explained by a greater titre of carboxyesterase, which specifically hyrolyses the carboxyester moiety of the malathion molecule. As a result, resistant insects present lower levels of malaoxon than susceptible insects after exposure to malathion (MATSUMARA & BROWN, 1961; MATSUMARA & HOGENDIJK, 1964, cited by ZETTLER, 1974b). This type of resistance can be overcome by the coapplication of malathion with the synergist tripenyl phosphate (TPP), a carboxyesterase inhibitor (DYTE & ROWLANDS, 1968). The non-specific type of resistance to malathion, in which the insects show cross resistance to other insecticides, cannot be overcome by T.P.P. (ROWLANDS, 1975, cited by PRICE, 1984).

CHAMP & CAMPBELL-BROWN (1970) recorded the non-specific type of resistance to malathion in T. castaneum associated with peanuts in Australia, which also showed cross resistance to several insecticides including fenitrothion. HALISCAK & BEEMAN (1983) also found some populations of T. castaneum in the United States with non-suppressible resistance to malathion when exposed to insecticide plus TPP.

In the 1972/73 survey carried out by FAO, seven of the eight brazilian populations of T. castaneum and the brazilian population of T. confusum tested exhibited the non-specific type of resistance (CHAMP & DYTE, 1976).

In a resistance survey to malathion carried out in the State of São Paulo (SARTORI et alii - in press), resistance was observed in 79%, 100% and 100% of the tested populations of S. oryzae, R. dominica and T. castaneum, respectively. It was also observed that 71% of the populations of T. castaneum exhibited specific and non-specific types of resistance to malathion while resistance in R. dominica was of the specific type.

Pirimiphos-methyl and fenitrothion have been used in Brazil to replace malathion in the control of storage grain insects. Thus a survey to verify the presence of resistance to these insecticides is essential in order to evaluate their efficiency, as well as to be prepared for possible failures in insect control.

The object of this research was to carry out this survey with the principal stored grain coleoptera, in order to provide data for a suitable control during storage.

MATERIAL AND METHODS

Samples of infested grain were collected from grain storage facilities located in different regions of Brazil. Up to the present moment samples have been collected in the States of São Paulo (SP), Rio Grande do Sul (RS), Goiás (GO), Santa Catarina (SC), Acre (AC) and Rondonia (RO). The insects S. oryzae,

S. zeamais, R. dominica e T. castaneum were separated and reared on specific media according to MILLER et alii (1969). These populations were submitted to resistance tests to malathion, pirimiphos-methyl and fenitrothion. The populations of R. dominica and T. castaneum were also submitted to tests to determine the type of resistance to malathion. The populations from the State of São Paulo had already been tested for resistance and type of resistance to malathion in a survey carried out from 1986 to 1988 (SARTORI et alii, in press).

The assessment of resistant and susceptible insects was undertaken by means of bioassays, exposing adults to filter papers impregnated with insecticide at the discriminating dose for the appropriate period, according to the FAO Standard Method (FAO, 1975). The discriminating dose is that which expected to Kill or "Knock down" all susceptible specimens. The discriminating concentrations in percentage (weight of insecticide/vol. risella oil) and the exposure times for malathion, standardized by FAO (FAO, 1975) and for pirimiphos-methyl and fenitrothion, according to the Natural Resources Institute - England (TAYLOR, 1989 - personal communication) are:

Sitophilus spp.	malathion -	1.5
exposure time: 6 h	pirimiphos-methyl -	2.0
	fenitrothion -	0.8
R. dominica	malathion -	2.5
exposure time: 24 h	pirimiphos-methyl -	3.0
	fenitrothion -	1.0
T. castaneum	malathion -	0.5
exposure time: 5 h	pirimiphos-methyl -	0.7
	fenitrothion -	0.4

To determine the type of resistance (specific or not), R. dominica and T. castaneum were exposed to papers impregnated with a misture of malathion plus triphenyl phosphate (1:5) at the discriminating dose, since specific resistance is suppressed in these species by this synergist.

Each biossay was carried out in duplicate, using 40 insects per trial. The tests were carried out at 26.0 ± 2.0ºC and 70.0 ± 5% R.H. After the exposure time, the live, dead and knocked down insects were counted and the mortality corrected according to ABBOTT (1925). The knocked down insects were considered as dead.

RESULTS AND DISCUSSION

The results of the tests with S. oryzae, S. zeamais, R. dominica and T. castaneum are shown, respectively, in Tables 1 to 4.

TABLE 1. Response (mortality %) of populations of *S. oryzae* exposed to the discriminating doses of malathion, pirimiphos-methyl and fenitrothion.

Local	Grain	Sampling Date	Malathion	Pirimiphos Methyl	Fenitrothion
Assis (SP)	wheat	1986	100	100	100
Assis (SP)	corn	1986	100	100	100
Sumaré (SP)	wheat	1986	100	100	100
Tatuí (SP)	wheat	1986	49	100	100
São Joaq. da Barra (SP)	wheat	1986	36	100	100
São Manuel (SP)	corn	1987	91	100	100
São Manuel (SP)	rice	1987	46	86	100
Ourinhos (SP)	corn	1988	90	85	100
Palmital (SP)	corn	1988	38	100	100
Palmital (SP)	wheat	1988	39	100	100
Assis (SP)	wheat	1988	79	100	100
Assis (SP)	corn	1988	83	100	100
Assis (SP)	rice	1988	96	100	100
Tupã (SP)	wheat	1988	44	100	100
Tangará (SC)	wheat	1989	100	100	100
Estrela (RS)	wheat	1989	100	100	100
Cachoeira do Sul (RS)	wheat	1989	99	100	100
Porto Alegre (RS)	wheat	1989	96	100	100
Caçapaca (RS)	wheat	1989	100	100	100
Porto Velho (RO)	rice	1989	95	90	93

Thirteen (65%) of the twenty populations of *S. oryzae* tested showed resistance to malathion, there being an indication of cross resistance to pirimiphos-methyl in two populations (10%) and to both insecticides in one (5%).

In the world survey carried out by FAO in 1972-73 (CHAMP & DYTE, 1976) no resistance was observed by the brazilian populations of *S. oryzae*. However, in this survey only four populations from Brazil were tested, one being from Goiás, two from Rio Grande do Sul and one from São Paulo.

TABLE 2. Response (mortality %) of populations of *S. zeamais* exposed to the discriminating doses of malathion, pirimiphos-methyl and fenitrothion.

Local	Grain	Sampling Date	Malathion	Pirimiphos Methyl	Fenitrothion
Morrinhos (GO)	corn	1989	100	100	99
Edeia (GO)	corn	1989	100	100	100
Rio das Antas (SC)	corn	1989	100	100	100
Witmarsun (SC)	corn	1989	100	100	100
Maravilha (SC)	corn	1989	100	100	100
D. Emma (SC)	corn	1989	100	100	100
S.M. D'Oeste (SC)	corn	1989	100	100	100
Cachoeira do Sul (RS)	corn	1989	100	100	100

The eight populations of *S. zeamais* tested were shown to be susceptible to the three insecticides.

In the world survey carried out by FAO (CHAMP & DYTE, 1976) there was also no resistance shown by the nineteen brazilian populations of *S. zeamais* collected in the States of Goiás, Rio de Janeiro, Rio Grande do Sul and São Paulo.

TABLE 3. Response (mortality %) of populations of *Rhyzopertha dominica* exposed to the discriminating doses of malathion + triphenyl phosphate (TPP), pirimiphos-methyl and fenitrothion.

Local	Grain	Sampling date	Malathion	Malathion + TPP	Pirimiphos-methyl	Fenitrothion
Assis (SP)	wheat	1986	73	100	100	100
São Joaquim da Barra (SP)	wheat	1986	66	100	100	96
Araçatuba (SP)	corn	1987	97	100	100	100
Araçatuba (SP)	rice	1987	80	100	100	100
São Manuel (SP)	rice	1987	36	100	100	100
Araraquara (SP)	corn	1987	85	100	100	100
Presidente Prudente (SP)	rice	1988	85	100	100	100
Marília (SP)	corn	1988	100	100	100	100
Palmital (SP)	corn	1988	67	100	100	71
Assis (SP)	rice	1988	91	100	100	100
Goiatuba (GO)	rice	1988	75	100	100	100
Santa Helena (GO)	sorghum	1989	100	100	100	100
Goiânia (GO)	rice	1989	100	100	100	100
Itaberaci (GO)	rice	1989	100	100	100	100
Cachoeira do Sul (RS)	wheat	1989	91	100	91	100
Cachoeira do Sul (RS)	rice	1989	100	100	100	100
Camaquã (RS)	rice	1989	93	100	100	100
Humaitá (AC)	rice	1989	73	100	100	100
Rio Branco (AC)	corn	1989	92	100	100	100
Porto Velho (RO)	rice	1989	100	100	100	100

Fourteen (70%) of the twenty populations of *R. dominica* tested showed the specific type of resistance to malathion, there being 100% mortality when they were exposed to malathion synergised with triphenyl phosphate. However, cross resistance to pirimiphos-methyl was indicated in one population (5%) and to fenitrothion in two populations 10%. In monitoring testes some TPP synergism would be expected in populations of *R. dominica* with non-specific OP resistance so that these would not be recognised as such unless their resistance level was fairly high. (CHAMP & DYTE, 1976).

It is likely that another mechanism of resistance is involved in addition to carboxyesterase, contributing to the resistance in these populations. Indications of other resistance mechanisms besides carboxyesterase, as a non specific esterases, oxidative enzymes and glutatione - S-transferase activity were noted in a population of *R. dominica* (ATTIA, 1981).

TABLE 4. Response (mortability %) of populations of *T. castaneum* exposed to the discriminating doses of malathion, malathion + triphenyl-phosphate (TPP), pirimiphos-methyl and fenitrothion.

Local	Grain	Sampling date	Malathion	Malathion + TPP	Pirimiphos-methyl	Fenitrothion
Ribeirão Preto (SP)	corn	1986	0	100	100	100
São Joaquim da Barra (SP)	wheat	1986	0	45	93	96
Ourinhos (SP)	wheat	1986	9	80	91	60
Araçatuba (SP)	rice	1987	0	91	100	100
Araçatuba (SP)	corn	1987	1	8	100	81
São Manuel (SP)	rice	1987	0	59	100	100
Baurú (SP)	corn	1987	0	90	100	100
Baurú (SP)	rice	1988	6	81	100	100
Assis (SP)	rice	1988	4	96	100	100
Ourinhos (SP)	corn	1988	0	100	100	100
Tupã (SP)	corn	1988	3	100	100	100
Tupã (SP)	wheat	1988	0	100	94	86
Palmital (SP)	wheat	1988	3	24	91	51
Palmital (SP)	corn	1988	1	85	100	100
Goiatuba (GO)	rice	1988	0	7	59	92
Maurilândia (GO)	corn	1989	0	89	100	100
Maurilândia (GO)	sorghum	1989	0	50	100	92
Edéia (GO)	corn	1989	1	67	92	100
Itumbiara (GO)	rice	1989	0	75	100	100
Itaberaci (GO)	corn	1989	0	96	100	100
Santa Helena (GO)	sorghum	1989	17	78	100	100
Morrinhos (GO)	corn	1989	0	0	59	84
Cachoeira do Sul (RS)	wheat	1989	34	52	100	100
Humaitá (AC)	rice	1989	12	25	95	92
JI Paraná (RO)	rice	1989	1	19	89	79

The twenty-five populations of *T. castaneum* tested showed resistance to malathion. There was an indication of the specific type of resistance in four (16%) of these populations. Twenty populations (80%) showed indications of specific and non-specific types of resistance to malathion, there being synergism, but the resistance not being suppressed by exposure to malathion plus the synergist triphenyl-phosphate. One (4%) population (Morrinhos) showed the non-specific type of resistance, synergism not being observed when the insects were exposed to the insecticide plus the synergist.

There was an indication of cross-resistance to pirimiphos-methyl in one population (4%), to fenitrothion in two (8%) and to both insecticides in eight (32%).

Indication of cross resistance to pirimiphos-methyl and fenitrothion was observed in one populations that showed specific type of resistance (Tupã wheat). Is is likely that another mechanism of resistance is involved in addition to carboxyesterase, contributing to the resistance in this population despite of the indication of the specific type of resistance.

CONCLUSIONS

The results suggest that:

- Resistance to malathion in populations of S. oryzae, R. dominica and T. castaneum is widespread in Brazil, mainly in the State of São Paulo, where resistance was shown in 79%, 90% and 100% respectively of the populations tested.
- S. zeamais has not developed resistance to the three insecticides.
- Of the species tested T. castaneum showed the greatest ability to develop resistance to these insecticides with 44% of the populations tested exhibiting cross resistance to either one or both of the insecticides pirimiphos-methyl and fenitrothion. S. oryzae and R. dominica showed indications of cross resistance in 15% of the populations.

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RESISTANCE AU MALATHION, AU PIRIMIPHOS-METHYL ET AU FENITROTHION CHEZ LES COLEOPTERES DES GRAINS STOCKES

Ivania Athié PACHECO, Maria Regina SARTORI et Sheila BOLONHEZI

ITAL, Instituto de Tecnologia de Alimentos
C.P. 139, Campinas, SP, Brasil 13073

RESUME

Les objectifs de cette publication sont : 1) de vérifier l'apparition du phénomène de résistance au malathion, au pirimiphos-méthyl et au fénitrothion chez les populations d'insectes des grains stockés ; 2) d'obtenir des données pouvant contribuer à une lutte adéquate pendant le stockage, réduisant ainsi les pertes.

Les populations de *Sitophilus oryzae*, *S. zeamais*, *Tribolium castaneum* et *Rhyzopertha dominica* ont été récoltées dans des installations de stockage situées dans diverses régions du pays et, transmises aux laboratoires du Département du Stockage du Grain de l'ITAL où elles ont subi le test du papier buvard imprégné, suivant les normes de la FAO.

Jusqu'à présent, dans les analyses entreprises, on a découvert une indication de résistance au malathion et au pirimiphos-méthyl dans 65 % et 10 % des populations de *S. oryzae*, respectivement. Aucune indication de résistance au fénitrothion chez *S. oryzae* n'a été découverte. *S. zeamais*, n'a montré aucune résistance vis-à-vis de tous les insecticides étudiés.

Pour *T. castaneum*, 100 % de la population étudiée s'est montrée résistante au malathion (83 % du type non spécifique), 35 % au pirimiphos-méthyl et 39 % au fénitrothion.

Pour *T. castaneum*, 79 % de la population étudiée s'est montrée résistante au malathion, 7 % au pirimiphos-méthyl et 14 % au fénitrothion.