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Resistance detection of *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae) to organophosphorous and pyrethroids insecticides

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Abstract

To verify if resistance is responsible for the low efficiency of the protectant insecticides used to control *Oryzaephilus surinamensis* (L.), bioassays were carried out with twelve strains of this species, originated from different localities of southern Brazil. The standard technique recommended by F.A.O. using the insecticide impregnated filter paper method was used with the organophosphorous insecticides fenitrothion and pirimiphos-methyl, and pyrethroids deltamethrin and bifenthrin. The strains OS12 and OS10 showed resistance levels of, 11.6 and 12.7 times to the organophosphorous insecticide fenitrothion, respectively. The OS3 and OS10 strains were 21.1 and 23.5 times more resistant to the pyrethroid deltamethrin than the susceptible strain, respectively. The study proved that strains with historical of continuous use of insecticides showed higher level of resistance to insecticides tested, requiring an appropriate management for its control.

Key words: Oryzaephilus surinamensis, Coleoptera, resistance, organophosphorous, pyrethroid

Introduction

The *Oryzaephilus surinamensis* (L.) species is a stored grains pest that can damage a mass of grain significantly when population density is high, which leads to the wide use of insecticides by warehouses.

The success of a treatment with residual insecticide depends, among other factors, on the population resistance status (Barson et al., 1992). According to Collins (1985), one of the biggest disadvantages of the chemical grain protectors is the selective pressure on the insect populations, favoring the development of the resistance.

The resistance of pests to insecticides is an example of evolution of the species, demonstrating how they can survive and change physiologically under pressure of the chemists that select them genetically (Lorini and Beckel, 2002). For example, the resistance of the stored grain pest *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) to the pyrethroid insecticide deltamethrin (Lorini and Galley 1999; 2001) and the cross resistance of the same pest to the insecticides pirimiphos-methyl, clorpiriphos-methyl and permethrin (Lorini and Galley, 2001) resulted from the association of the metabolic mechanisms of resistance and reduction of nervous system sensibility.

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In agreement with Watson and Barson (1996), the incidence and the degree of resistance of *O. surinamensis* to insecticides has been increasing since the resistance detection to the insecticide lindane, in the beginning of the decade of 1970. Populations of the pest, in several countries, have been developing resistance to the insecticides malathion, fenitrothion, clorpiriphos-methyl and pirimiphos-methyl, (Brun and Attia, 1983; Herron, 1990; Collins et al., 1993).

According to Taylor and Georghiou (1982), simulation models indicate that conditions like the wide use of persistent insecticides against insect populations, with little or no migration of susceptible insects, are ideal for the development of the resistance.

This situation has been observed in warehouses of the southern region of Brazil, where *O. surinamensis* has been occurring in practically all storage facilities and causing serious damage to the storage of grain. The species has not been answering significantly to chemical treatments, and is one of the first to colonize the mass of grain after the application of insecticides (Lorini, 2001).

Another aggravating factor is the availability of only four insecticides registered for the control of stored grain pests: two organophosphorous (fenitrothion and pirimiphos-methyl) and two pyrethroids (deltamethrin and bifenthrin) (Lorini, 2001).

In spite of the low effectiveness of the insecticides in the control of *O. surinamensis*, in Brazil, there are still no studies to evaluate if it is a case of resistance or faults in the protectors application. Thus, the objective of this work was to investigate the resistance of populations of *O. surinamensis* collected in warehouses of the southern region, to the organophosphorous and pyrethroids insecticides registered for use in the control of the pest.

Material and methods

Twelve populations of *O. surinamensis*, collected in storage facilities of grains and identified according to its origin and date of collection were tested (Table 1).

For each population of *O. surinamensis* used in the experiment, the historical of exposure to the pyrethroids and organophosphorous insecticides was researched. The individuals of the OS1 population were collected in one storage

Table 1 Origin and year of collection of the *Oryzaephilus surinamensis* populations used in the bioassays for the verification of resistance to the organophosphorous and pyrethroids insecticides, in laboratory.

Population	Origin	Year of collection
OS1	Passo Fundo – RS	1998
OS2	Ubiratã – PR	1998
OS3	Santo Ângelo – RS	1998
OS4	Saldanha Marinho – RS	1998
OS5	Assai – PR	2000
OS6	Kaloré – PR	2000
OS7	Marau – RS	2001
OS9	Ubiratã – PR	2001
OS10	Assis Chateaubriand – PR	2001
OS11	Mandaguari – PR	2002
OS12	Maringá – PR	2002
OS13	Videira – SC	2002

facility where the grain received only treatment with gas phosphine.

The OS3, OS7 and OS12 populations were submitted to the applications of pyrethroids insecticides for 10, 3 and 5 years, respectively. The other populations received applications of mixtures of pyrethroids and organophosphorous insecticides along the years in the stored grain facilities where they were collected, as it follows: the OS2 population received sporadic applications of mixtures, OS13 was submitted to selection pressure by mixtures for 3 years, OS4 and OS9 populations for 5 years, OS6 for 8 years and OS5, OS10 and OS11 populations for 10 years before the collection.

All the populations, from the time of the collection, were maintained at the Entomology Laboratory of Embrapa Wheat, in Passo Fundo, RS, and because of that, they received the generation designation F_{n+1}, indicating the first generation obtained in laboratory.

The insects used in the experiments were maintained in wheat grain in a room at 25 ± 0.5 ºC temperature and 65 ± 5 % relative humidity.

Four insecticides registered in Brazil for the control of stored grain pests, were used in the experiment as follows: organophosphorous, fenitrothion 500 g a.i. /L (Sumigran 500 EC) and pirimiphos-methyl 500 g a.i. /L (Actellic 500 EC); and pyrethroids, deltamethrin 25 g a.i. /L (Decis 25 EC) and bifenthrin 25 g a.i. /L (Brigade 25 EC).

For the development of the bioassays the standard technique recommended by F.A.O. was adopted - Method nº 15 (Anonymous, 1974). Twelve populations were tested with the insecticides fenitrothion and deltamethrin, while the populations OS1, OS2, OS3, OS4 and OS5 were tested just with the insecticides pirimiphos-methyl and bifenthrin.

Each population was submitted to five treatments, replicated four times, with each insecticide and a control, just treated with solvent. Each insecticide was diluted in petroleum ether in the requested concentrations, and 1.0 ml of the concentration was applied on filter paper with 9 cm of diameter. These were maintained in a ventilated room during approximately one hour, for evaporation of the solvent, before being released in the Petri plates. Later, 10 adult insects of *O. surinamensis* with 1-20 days of age, without being sexed were placed in each repetition. The experiment was maintained in a room at 25 ± 0.5 ºC temperature and 65 ± 5 % relative humidity.

The number of dead adults was counted 24 hours after the treatment, to establish the lethal concentrations (CL_{50}) of each population, being considered dead the insects that could not walk normally during a period of observation of 2 minutes.

For the determination of CL_{50} for each population, the mortality results of the bioassays were analyzed by the statistical program GLIM, Royal Statistical Society, version 3.77 (Crawley, 1993).

Results and discussion

The bioassays results in filter paper treated with the organophosphorous and pyrethroids insecticides showed that the OS1 population, like the historical already indicated, was the most susceptible (Tables 2, 4 and 5). While, the results of the bioassays with the pyrethroid deltamethrin, determined that the OS13 population was the most susceptible to this kind of treatment (Table 3).

The bioassay with fenitrothion showed that the OS2 and OS3 populations did not present significant differences with CL_{50} of the OS1 population, being therefore considered susceptible to that insecticide, since the CL_{50} value of the OS1 population represents the normal level of tolerance of the species to that insecticide (Table 2). The individuals of the OS2 population received sporadic applications of organophosphorous and pyrethroids insecticides mixtures, while the OS3 population suffered selection pressure with just pyrethroids insecticides, justifying the found result.

Table 2 Values of CL_{50} (mg/cm^2) for adults of populations of *Oryzaephilus surinamensis* (L.) exposed to paper filter treated with fenitrothion for the determination of the resistance factor, at 25 ± 0.5 ºC temperature and 65 ± 5 % relative humidity. Passo Fundo, RS, 2003.

Population/ Generation	CL_{50} (95 % C.l.)*	a	SE_a	b	SEb	FR
OS1 (Fn+5)	0.4105 (0.3205-0.5331) A	1.180	0.2469	3,052	0.4366	-
OS3 (Fn+6)	0.6787 (0.4563-1.217) AB	0.2925	0.2023	1,737	0.3775	1.6
OS2 (Fn+7)	0.7595 (0.5343-1.275) AB	0.2416	0.2034	2,022	0.3906	1.8
OS5 (Fn+3)	0.9466 (0.066-1.439) BC	0.06506	0.2051	2,729	0.4680	2.3
OS9 (F^0)	1,463 (1.011-2.835) CD	-0.4148	0.2047	2,509	0.4947	3.5
OS13 (F^0)	1,882 (1.493-2.293) D	-1.233	0.3046	4,490	0.6492	4.5
OS11 (F^0)	2,546 (2.106-3.046) E	-2.101	0.3648	5,177	0.6939	6.2
OS7 (F^0)	2,556 (2.130-3.040) EF	-2.235	0.3802	5,484	0.7348	6.2
OS6 (F^0)	2,911 (2.462-3.428) EF	-2.858	0.4463	6,160	0.8187	7.1
OS4 (Fn+5)	3,4880 (2.608-4.651) FG	-1.422	0.2772	2,620	0.4126	8.5
OS12 (F^0)	4,780 (4.026-5,694) GH	-3.825	0.5352	5,629	0.7363	11.6
OS1O (F^0)	5,235 (4.393-6.273) H	-3.879	0.5386	5,396	0.7096	12.7

* Values followed by the same letter are not significantly different (P>0.05) by F test.

a = linear coefficient; b = angular coefficient; SE = Standard error

RF = Resistance Factor (any CL_{50} divided by CL_{50} of the population OS1)

Table 3 Values of CL_{50} (mg/cm^2) for adults of populations of *Oryzaephilus surinamensis* (L.) exposed to paper filter treated with deltamethrin for the determination of the resistance factor, at 25 ± 0.5 ºC temperature and 65 ± 5 % relative humidity. Passo Fundo, RS, 2003.

Population/ Generation	CL_{50} (95 % C.l.)*	a	SE_a	b	SEb	RF
OS13 (F^0)	3.010 (2.127-4.084) A	-1.136	0.2746	2,374	0.4071	-
OS1 (Fn+4)	15.73 (8.439-22.92) B	-2.419	0.6283	2,021	0.4101	5.2
OS2 (Fn+6)	16.22 (10.33-22.06) B	-3.106	0.6746	2,567	0.4537	5.3
OS4 (Fn+4)	21.86 (15.46-28.75) B	-3.685	0.6816	2,751	0.4470	7.2
OS11 (F^0)	22.93 (16.39-30.09) BC	-3.764	0.6827	2,766	0.4453	7.6
OS9 (F^0)	23.95 (16.86-31.98) BC	-3.504	0.6540	2,540	0.4248	7.95
OS12 (F^0)	31.13 (23.69-40.07) BCD	-4.536	0.7184	3,038	0.4533	10.34
OS5 (Fn+3)	36.49 (26.08-50.40) BCDE	-3.576	0.6507	2,289	0.3989	12.1
OS6 (F^0)	41.82 (32.61-53.89) CDE	-5.138	0.7603	3,169	0.4606	13.8
OS7 (F^0)	47.61 (37.28-62.10) DE	-5.216	0.7462	3,109	0.4476	15.8
OS3 (Fn+5)	63.55 (49.81-84.45) DE	-5.752	0.8342	3,190	0.4775	21.1
OS10 (F^0)	70.74 (55.36-95.20) E	-5.916	0.8605	3,198	0.4860	23.5

* Values followed by the same letter are not significantly different (P>0.05) by F test.

a = linear coefficient; b = angular coefficient; SE = Standard error

RF = Resistance Factor (any CL_{50} divided by CL_{50} of the population OS1)

Table 4 Values of CL_{50} (mg/cm^2) for adults of populations of *Oryzaephilus surinamensis* (L.) exposed to paper filter treated with pirimiphos-methyl for the determination of the resistance factor, at 25 ± 0.5 ºC temperature and 65 ± 5 % relative humidity. Passo Fundo, RS, 2003.

Population/ Generation	CL_{50} (95 % C.l.)*	a	SE_a	b	SEb	RF
OS1 (Fn+5)	0.3543 (0.2746-0.4536) A	1.432	0.2611	3,177	0.4617	-
OS2 (Fn+7)	0.5837 (0.4534-0.7844) B	0.6927	0.2219	2,963	0.4435	1.6
OS3(Fn+6)	0.6371 (0.4718-0.9286) B	0.4735	0.2103	2,418	0.4166	1.8
OS5 (Fn+3)	0.7305 (0.5833-0.9541) B	0.4947	0.2206	3,628	0.5313	2.0
OS4 (Fn+4)	0.9416 (0.7131-1.3880) B	0.07592	0.2063	2,905	0.4845	2.6

* Values followed by the same letter are not significantly different (P>0.05) by F test.

a = linear coefficient; b = angular coefficient; SE = Standard error

RF = Resistance Factor (any CL_{50} divided by CL_{50} of the population OS1)

Table 5 Values of CL_{50} (mg/cm^2) for adults of populations of *Oryzaephilus surinamensis* (L.) exposed to paper filter treated with bifenthrin for the determination of the resistance factor, at 25 ± 0.5 ºC temperature and 65 ± 5 % relative humidity. Passo Fundo, RS, 2003.

Population/ Generation	CL_{50} (95 % C.l.)*	a	SE_a	b	SEb	RF
OS1 (Fn+4)	2.686 (2.024-3.446) A	-1.321	0.2889	3,078	0.4623	-
OS3 (Fn+5)	6.358 (4.807-8.944) B	-2.129	0.3394	2,651	0.4332	2.3
OS5 (Fn+3)	6.668 (5.286-8.745) B	-2.822	0.4073	3,425	0.5025	2.4
OS4 (Fn+4)	7.950 (6.569-9.988) B	-4.023	0.4140	4,468	0.5005	2.9
OS2 (Fn+6)	11.45 (7.886-21.59) B	-2.311	0.3618	2,183	0.4335	4.2

* Values followed by the same letter are not significantly different (P>0.05) by F test.

a = linear coefficient; b = angular coefficient; SE = Standard error

RF = Resistance Factor (any CL_{50} divided by CL_{50} of the population OS1)

The OS10 and OS12 populations presented the largest resistance factors to fenitrothion: 12.7 and 11.6 times, respectively. The other populations, OS5, OS9, OS13, OS11, OS7, OS6 and OS4 exhibited resistance factors of up to 8.5 times, when compared to the susceptible population (Table 2).

The results found for the bioassays with the pyrethroid deltamethrin showed that the susceptible population, OS13, differed statistically from the other populations (Table 3), and that the OS10 population presented a factor of resistance of 23.5 times when compared to the OS13 population. This result is justified by the fact that the storage unit in which the insects of the OS10 population were collected presented a historical of applications of mixtures of organophosphorous and pyrethroids insecticides for, at least, ten years.

The results of the determination of the resistance of the populations of *O. surinamensis* to the pirimiphos-methyl organophosphorous insecticide showed that the OS2, OS3, OS4 and OS5 populations differed significantly from the susceptible population OS1, however they didn't differ statistically amongst themselves (Table 4). Also, it is possible, in this case, to infer that resistance is beginning to be expressed for the

same reason as the long exposure to insecticides already mentioned.

Similar results to the previous one was observed in bioassays with the pyrethroid bifenthrin insecticide, where the OS2, OS3, OS4 and OS5 populations differed significantly from the susceptible population OS1, but they didn't differ statistically amongst themselves (Table 5).

This result can be indicating an initial level of resistance to bifenthrin since, with the exception of the OS2 population that received sporadic applications of organofosforados and piretróides insecticides, the other populations, OS3, OS4 and OS5, received applications of piretróides and organofosforados insecticides for up to ten years before the collection.

The results of the tests with the four insecticides confirm the studies of Collins (1985), indicating that infestations in warehouses are the result of grain already infested or of residual populations of *O. surinamensis* in grocery stores and possibly being continually submitted to the insecticides selection pressure.

As the report indicated, the individuals of all of the populations were submitted to a smaller or larger degree of selection pressure by insecticides and the resistance of those populations was predicted, considering the control flaws evidenced in most of the storage units.

Besides, the value of b (angular coefficient), observed in the bioassays analysis in all populations, it indicates a considerable heterogeneity of these individuals to the tested insecticides (Tables 2, 3, 4 and 5). This suggests a great potential for the development of larger levels of resistance with the continuity of selection pressure (Ding et al., 2002).

However, Muggleton (1983), which worked with populations of *O. surinamensis* resistant to malathion, indicated that the polymorphism for the resistance in this insecticide is transitory, because, in the absence of the insecticide, the resistant individuals' frequency in the population refused quickly.

Although similar data has not been verified in this work for the tested insecticides, possibly, the different populations of *O. surinamensis* existent in storage units recover the susceptibility quickly if the selection pressure by insecticides is interrupted and with the immigration of new populations in infested grain.

Concluding, as the species *O. surinamensis* still had not been studied in Brazil with regards to insecticide resistance, this work demonstrated that the species presents varied levels of resistance to the tested insecticides, in agreement with the origin and the historical of exposure to the active ingredients, evidencing that the use of insecticides to control stored grain pests needs to be managed carefully in the programs of resistance handling.

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