CROP PROTECTION

Pirimiphos-Methyl Residues in Corn and Popcorn Grains and Some of their Processed Products and the Insecticide Action on the Control of *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae)[1]

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Neotropical Entomology 32(4):707-711 (2003)

Resíduos de Pirimifós-Metil em Milho, Milho Pipoca, em Alguns de seus Produtos Derivados e Ação no Controle de *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae)

RESUMO - Foi avaliada a degradação/persistência dos resíduos do inseticida organofosforado pirimifós-metil em grãos e produtos derivados de milho (farelo, canjica e farinha de milho) e de milho pipoca (pipoca), bem como a ação residual do inseticida no controle do gorgulho do milho, *Sitophilus zeamais* Mots. Os grãos foram tratados com a concentração de 12 mg.kg^{-1} (ppm) de ingrediente ativo para controle do gorgulho. As amostras foram tomadas logo após o tratamento e também aos 15, 30, 60, 120 e 240 dias após o tratamento. A determinação quantitativa foi feita por cromatografia em fase gasosa, usando-se um detector fotométrico de chama. Para os estudos sobre a ação residual de pirimifós-metil, amostras de ambos os tipos de grãos foram tomadas aos 15 e 30 dias após o tratamento e subseqüentemente a intervalos mensais pelo período de um ano. Grãos tratados e não-tratados (testemunha) foram infestados com adultos do gorgulho. A mortalidade foi avaliada 15 dias após a exposição. Os resíduos de pirimifós-metil não foram persistentes em ambos os tipos de grãos nem nos seus produtos derivados. Os resíduos decresceram 5-8 vezes no farelo de milho e na pipoca. Na canjica e na farinha de milho eles foram estáveis, porém os níveis foram baixos. Resíduos maiores no farelo do que nos grãos estão relacionados com o maior teor de óleo no produto processado. O inseticida manteve-se eficiente contra a praga por todo período de observação (1 ano) para os dois tipos de grãos.

PALAVRAS-CHAVE: Resíduo de agrotóxicos, degradação/persistência, gorgulho-do-milho, grãos armazenados

ABSTRACT - The degradation/persistence of residues of the organophosphorus insecticide pirimiphos-methyl was determined in corn and popcorn grains and in some of their processed products such as grain, bran, hominy and corn flour from corn and prepared popcorn and grain from popcorn. The action of the insecticide on the control of the corn weevil, *Sitophilus zeamais* Mots., was also evaluated. The grains were treated with the concentration of 12 mg.kg^{-1} (ppm) a.i. pirimiphos-methyl for the control of the weevils. Samples were taken at zero, 15, 30, 60, 120 and 240 days after treatment. Quantitative measurements were made by gas chromatography, by using a flame photometric detector. For pirimiphos-methyl residual action studies, samples of both types of grain were taken at 15, 30 days after treatment and subsequently at monthly intervals up to one year. Treated and non-treated (control) grains were infested with weevil adults. Mortality was checked 15 days after exposure. Pirimiphos-methyl residues were not persistent in either types of grains or their processed products. Residues dropped 5- 8-fold in corn bran and popcorn. They were stable in hominy and corn flour, but the concentration levels were low. Higher residues in corn bran than in grain are related to the higher oil content in that processed product. The insecticide remained effective against the pest over the entire period of observation (1 year) for both types of grains.

KEY WORDS: Pesticide residue, degradation/persistence, corn weevil

It is estimated according to FAO data that 10% of world grain production is lost annually because of insect attack in warehouses; in Brazil losses are around 20% because of precarious storage conditions (Gallo *et al.* 2002).

This problem becomes more significant when the great importance of cereal grains in human nutrition is considered

and further, the fact that some of the public and private investments applied in grain production are lost post harvest.

Because of these problems caused by insects in warehouses, chemical substances have been used mainly to control these pests and to preserve the harvests but these insecticides may persist in the form of toxic residues in the treated grains and their consumption by man and animals, even in sub-lethal quantities, represents a potential risk to consumer health. The organophosphorus insecticide pirimiphos-methyl is much used among these insecticides for warehouse insect control.

Official control of pesticides in foods is generally based on the maximum residue levels (MRL) or tolerance and safety intervals established case by case. Some studies on the occurrence of residues in grains have been published in Brazil. Alleoni & Baptista (2001) after treating corn with the organophosphorus insecticide pirimiphos-methyl (6 and 12 $mg.kg^{-1}$) analyzed residues in the grains monthly for six months and reported residue half lives of 93-95 days, respectively, for the higher and lower concentrations. However, almost nothing is known about the occurrence of residues in the processed products and consequently about health risks. Processing usually reduces residues in industrialized products but in some cases increases them (Trevizan & Baptista 2000).

Studies on the effects of storage and some commercial processing techniques regarding residues in foods are part of the requirements for pesticide registration in many countries. The Joint Meeting Pesticide Residues (JMPR) FAO/OMS committee considers processing effects as part of their revision of pesticide residue data (Holland *et al.* 1994).

One of the most important pests that attack cereals in warehouses is the corn weevil *Sitophilus zeamais* Mots., because it has a high biotic potential, presents crossed infestation and attacks many hosts and both larvae and adults damage grains (Gallo *et al.* 2002).

The objective of this study was the assessment of the degradation/persistence of the organophosphorus insecticide pirimiphos-methyl residues in corn and popcorn grains and in some of their processed products and the effect of this insecticide on *S. zeamais* control.

Materials and Methods

Grain Treatments. Pirimiphos-methyl was applied on corn and popcorn grains to produce a theoretical concentration of 12 $mg.kg^{-1}$ (ppm). A complete randomized block design was used with two treatments (treated grains and control) and three replications; the commercial product used was Actellic 500 CE that contains 500g pirimiphos-methyl.L^{-1}.

The product was applied with a back pack sprayer at constant pressure with CO_2, using 60 ml insecticide emulsion (dilution 3:500 in water) for each 15 kg grains (1 L commercial product.ton^{-1}). For each treatment operation, the grains were spread in a thin layer on a plastic sheet; after application, the grains were mixed better by agitating the plastic sheet with vigorous movements. A control group was also included, conducted in the same manner, but only water was applied in the spraying. After the treatment, the grains were placed in plastic bags, kept open and stored at room temperature and humidity on the laboratory bench and sheltered from light.

Samples. The samples were taken at zero, 15, 30, 60, 120 and 240 days after treatment and the samples of the processed products were obtained at the same times. Corn and popcorn samples were analyzed and some of their processed products such as grain, bran, hominy and flour from corn and prepared popcorn and popcorn grains. The hominy and corn bran samples were obtained in a first step of the processing by using a small hominy pan; part of the hominy was ground to obtain the material for analysis. The corn flour was obtained from part of the hominy that was left immersed in water, which was changed daily, for three days, for later milling in an appropriate machine. The 'burst´ popcorn was prepared in the conventional way in an aluminum saucepan with a thin layer of oil on the bottom and heated over the stove; the popcorn obtained was then ground in a multiprocessor electrical appliance.

Two hundred and sixteen samples were analyzed (36 of each substrate) and 72 were obtained from grains and 144 from processed products (108 from the treatment and 108 controls).

Analytical Procedure. The analytical method was adapted from Ohlin (1998). For residues extraction, 10 g of the homogenized samples were taken (5 g for popcorn) and placed in Duran-Schott 200 ml flasks in which 50 ml acetone were added and shaken for about 15 min. at 360 cycles/min. After this operation, the extracts were centrifuged for 5 min. at 2,000 rpm to better separate the liquid phase from the materials in suspension. Then, 5 ml aliquots of supernatant, corresponding to 2 g of the original sample (1 g popcorn) were transferred to 50 ml polypropylene tubes and the extracts were concentrated under evaporation by means of air flow in a water bath at 45ºC.

Extracts clean-up was performed by liquid/liquid partition between saturated hexane with acetonitrile (8 ml) and acetonitrile saturated with hexane (25 ml) in the polypropylene tubes. After manual shaking and centrifuging for 5 min. at 2,500 rpm, the liquid was drained and the lower layer (acetonitrile) was transferred to other polypropylene tubes. In sequence, the extracts were again concentrated by evaporation in a water bath at 60ºC and re-suspended in 5 ml hexane. Clean-up was completed by solid phase extraction (SPE) column chromatography mounted in 10 ml hypodermic syringes containing packaging of 1 g silica gel and transferring the hexane extracts to these colunns; the elution was proceeded with a mixture of hexane and acetone (15 ml, 9/1 v/v) and the clean extracts were collected in other polypropylene tubes. Then, after futher concentration by evaporation in a water bath at 45ºC the pirimiphos-methyl residues were dissolved in 10 ml acetone and transferred to 15 ml graduated centrifuge tubes.

The quantitative determinations were made by gas chromatography, using a Hewlett Packard gas chromatograph, model 6890, equipped with a flame photometric detector (FPD) with a specific filter for phosphorus at 526 nm, megabore chromatographic column

HP-5 x 0.53 mm x 1.5 film thickness. The operational conditions were: temperatures: column = 190ºC, injetor = 250ºC, detector = 250ºC; gas flows (ml/min.): N_2 (carrier) = 10, N_2 (make-up) = 50; air = 110; H_2 = 150. Under these conditions, the pirimiphos-methyl retention time was approximately 2 min. The residues were quantified using a Varian integrator, model 4400, directly from the calibration curve constructed in the linearity range of the detector, by comparison of the samples chromatographic peaks areas and the ones of the external standards.

Validation of the Analytical Method. The analytical method used for grains and processed products was validated by substrate fortification studies with five levels and three replications for each level (15 samples fortified from each substrate), and results between 70-120% recovery were considered acceptable.

Thus the limits of quantification (LOQs) established were: corn grain and hominy 0.05 $mg.kg^{-1}$, bran and corn flour 0.1 $mg.kg^{-1}$, popcorn grain 0.1 $mg.kg^{-1}$ and prepared popcorn 0.05 $mg.kg^{-1}$. The recoveries for corn grain ranged from 75-112%, bran 75-108%, hominy 79-102%, corn flour 77-109%, popcorn grain 75-95% and prepared popcorn 75-92%. The linearity of the detector was highly satisfactory, in the range from 0.01 to 10 ng pirimiphos-methyl ($r^2 = 0.9904$).

Residual Action of Pirimiphos-Methyl in the Control of *S. zeamais*. To study corn weevil control with pirimiphos-methyl 15 days after treatment, samples of corn and popcorn grains were removed and an experiment was set up in a complete randomized design with two treatments for each type of grain (treated and control) with five replications. Forty grams of each type of grain were placed in plastic flasks, with a perforated lid to allow ventilation and each flask was infested with 40 adult unsexed, laboratory reared *S. zeamais*, then the flasks were closed with the lids and placed inside a closed cardboard box. The box was left at room temperature on a laboratory bench.

The mortality was assessed 15 days after the infestation considering immobile individuals as dead if unable to move even when stimulated by incandescent electric light, placed close to them. The mortality observed was corrected according to Abbott (1925) whenever there was death in the control plots.

After 30 days and subsequently at monthly intervals up to 12 months, an identical procedure was carried out to the one previously described for infestation and mortality assessment of the insect, totaling thirteen assessments.

The mortality data obtained in the treated and control grain treatments were submitted to the χ^2 test applied to non-parametric statistical analysis for each of the 13 assessments made.

Results and Discussion

Pirimiphos-Methyl Residues. Tables 1 and 2 show the results obtained from the corn and popcorn grain analyses and their processed products. All the 108 analyses corresponding to the control samples indicated lower residues than the respective LOQs of the method for the corresponding substrates.

Table 1. Pirimiphos-methyl residues in corn and processed products (mean of 3 replications).

Days after treatment	Substrate	Mean ($mg.kg^{-1}$) m ± sd	Residues concentration index
Zero	Grain	3.96 ± 0.15	1
	Bran	6.19 ± 0.67	1.56
	Hominy	0.07 ± 0.01	0.02
	Corn flour	0.13 ± 0.05	0.03
15	Grain	3.57 ± 0.10	1
	Bran	4.96 ± 0.43	1.39
	Hominy	0.05 ± 0.01	0.01
	Corn flour	< 0.1	-
30	Grain	3.30 ± 0.46	1
	Bran	3.97 ± 0.46	1.20
	Hominy	0.07 ± 0.03	0.02
	Corn flour	0.12 ± 0.05	0.04
60	Grain	1.44 ± 0.19	1
	Bran	2.91 ± 0.16	2.02
	Hominy	0.07 ± 0.02	0.05
	Corn flour	< 0.1	-
120	Grain	1.14 ± 0.21	1
	Bran	2.40 ± 0.04	2.11
	Hominy	0.07 ± 0.01	0.06
	Corn flour	< 0.1	-
240	Grain	0.59 ± 0.06	1
	Bran	1.14 ± 0.09	1.93
	Hominy	0.06 ± 0.01	0.10
	Corn flour	< 0.1	-

Table 2. Pirimiphos-methyl residues in popcorn grain and popcorn (mean of 3 replications).

Days after treatment	Substrate	Mean ($mg.kg^{-1}$) m ± s	Residues concentration index
Zero	Grain	2.15 ± 0.08	1
	Popcorn	1.91 ± 0.08	0.89
15	Grain	1.48 ± 0.11	1
	Popcorn	1.31 ± 0.06	0.89
30	Grain	1.05 ± 0.08	1
	Popcorn	1.20 ± 0.11	1.14
60	Grain	0.98 ± 0.11	1
	Popcorn	0.83 ± 0.05	0.85
120	Grain	0.75 ± 0.05	1
-	Popcorn	0.67 ± 0.08	0.89
240	Grain	0.38 ± 0.04	1
	Popcorn	0.25 ± 0.02	0.66

The recovery of the insecticide from day zero in corn was lower than the concentration intended at application (Table 1). About 4 mg.kg^{-1} (ppm) was recovered, what represented 33% of the amount of the active ingredient used in the application. La Hue (1975) also obtained low recoveries of this insecticide in corn treated with 12 mg.kg^{-1}. Pirimiphos-methyl can be considered highly volatile as it has a relative high vapor pressure (13mPa at 30°C) (Tomlin 1995). We may thus take this fact as the possible explanation for the low deposit indices and therefore, of recovery in the initial analysis (zero day).

Relative persistence of the insecticide in the grains was observed in the first 30 days after application, with approximately 17% reduction, particularly due to the fact that storage was in light-free conditions. Degradation was accentuated in the 30- to 60-day period, presenting a reduction of around 56%. Very similar results were obtained by Sgarbiero *et al*. (2002) in wheat and in some of its processed products, and by Sowumni & Fetuga (1985) who reported a 3.3-fold reduction in pirimiphos-methyl residues in corn 150 days after treatment.

Comparison of the results of the different substrates analyzed showed a more significant concentration of pirimiphos-methyl in the external part of the grains. This was indicated by the higher insecticide contents (about 70%) of the bran (relatively oil-rich), when compared to the grains, as can be observed by the concentration index (mean of 1.7 ± 0.37)(Table 1). Hominy, consisting basically of corn endosperm and the flour obtained from it, presented relatively low residue contents (about 3%).

Trevizan & Baptista (2001) worked with deltamethrin in corn grains and their processed products and obtained similar results with high insecticide concentration in the bran but very small quantities in the hominy and flour.

Insecticide recovery, also in the case of popcorn was low on application day because of the same consideration mentioned previously in the case of corn, and its residues were equally non-persistent, decreasing about 5.7 fold during the period (Table 2).

On prepared popcorn, a small reduction in the insecticide residues was detected compared with the grain (the values in popcorn represented 90% compared with the grain) that is in line with Singh and Chawla (1982).

Pirimiphos-Methyl Residual Action in *S. zeamais* Control. The mortality results obtained for *S. zeamais* adults in corn grains (Table 3 and Fig. 1) and in popcorn (Table 4 and Fig. 2) show that the insecticide was quite efficient in controlling

Table 3. *S. zeamais* mortality (%) in corn treated with pirimiphos-methyl for one year storage (mean of 5 replications).

Grain	Days after treatment												
	15	30	60	90	120	150	180	210	240	270	300	330	360
Treated (12 mg.kg^{-1})	100.0	100.0	100.0	100.0	100.0	100.0	99.5	99.0	96.0	97.5	95.5	92.0	73.0
Control	2.0	0.0	0.5	1.0	0.5	0.5	1.0	1.5	4.0	12.0	18.0	9.5	6.5
$\chi^{2\,(1)}$	384.3	400.0	396.1	392.1	396.1	396.1	388.1	380.3	338.6	295.1	244.7	272.3	184.3

(1) Value of χ^2 tabulated for LD = 1 e 0.01% probability = 10.83

Table 4. *S. zeamais* mortality (%) in popcorn treated with pirimiphos-methyl for one year storage (mean of 5 replications).

Grain	Days after treatment												
	15	30	60	90	120	150	180	210	240	270	300	330	360
Treated (12 mg.kg^{-1})	100.0	100.0	100.0	100.0	100.0	100.0	99.5	98.5	98.0	96.0	93.5	91.0	73.0
Control	3.0	0.5	2.0	5.0	1.5	0.5	6.0	7.5	6.0	10.0	11.5	1.5	5.0
$\chi^{2\,(1)}$	376.7	396.1	384.3	361.9	388.2	396.1	350.7	332.4	339.1	296.9	269.6	259.3	194.4

(1) Value of χ^2 tabulated for LD = 1 e 0.01% probability = 10.83

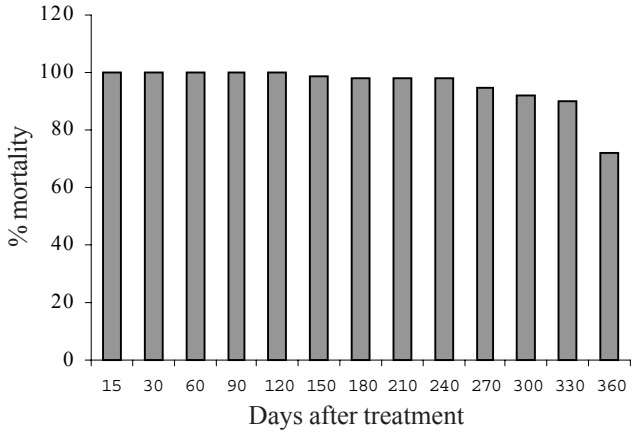

Figure 1. Adult *S. zeamais* mortality in exposure to corn treated with pirimiphos-methyl.

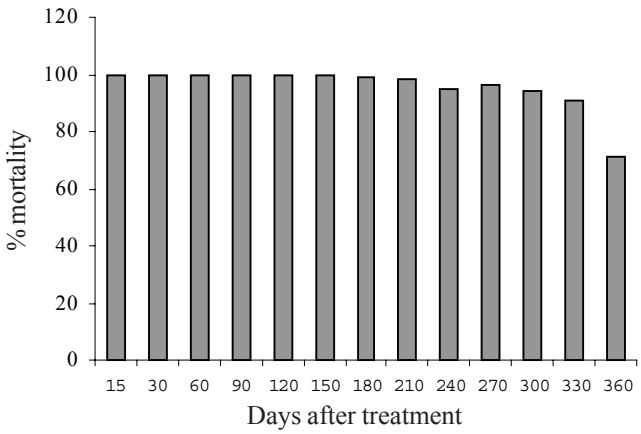

Figure 2. Adult *S. zeamais* mortality in exposure to popcorn treated with pirimiphos-methyl.

the pest, causing 100% mortality up to 150 days after the treatment, that remained over 90% until almost the end of the assessments in the one year period. These results are in line with Bitran *et al.* (1991) who reported high pirimiphos-methyl efficiency in *S. zeamais* control in corn grain for a period of 180 days. The residual effect of the insecticide was similar in both types of grain.

Acknowledgments

We thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support (Proc. FAPESP nº. 98/05989-9) and the scholarship to the first author (Proc. FAPESP nº. 98/05988-2). We also thank the Departamento de Produção Vegetal ESALQ/USP and the Fecularia Santa Teresinha for their collaboration in grain processing.

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Received 28/01/03. Accepted 03/10/03.