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Biological activity and persistence of pirimiphos-methyl applied to maize grain at different temperatures

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ABSTRACT

The expansion of dryeration may impose a further problem for insect control with protectants – the high grain temperatures during insecticide spraying. To assess the impact of this procedure on insecticide activity, maize grains at different temperatures (25, 30, 35, 40 and 45 °C) were sprayed with pirimiphos-methyl. Residue analyses were carried out every 30 days and insecticide biological activity towards *Sitophilus zeamais* and *Tribolium castaneum* was assessed every 15 days throughout the experimental period of 90 days. Insect mortality was evaluated after 48 h. Pirimiphos-methyl residue decreased with increased storage time and grain temperature during spraying. Similar trends were also observed for mortality of *S. zeamais* and *T. castaneum*, which dropped from around 100% for lower grain temperatures, shortly after spraying, to mortality values around 0% for higher temperatures and after 90 days of storage. These results indicate the drastic effect of grain temperatures during spraying, which compromises the efficiency of grain protectants for insect pest control on stored grains.

Key words: *Sitophilus zeamais, Tribolium castaneum*, organophosphate, residue, insect control

Eficácia biológica e persistência do pirimifós-metil aplicados sobre grãos de milho em diferentes temperaturas

RESUMO

A técnica de seca-aeração pode causar problema no controle de insetos devido à alta temperatura do grão durante o processo de pulverização do inseticida. Com este trabalho objetivou-se avaliar o efeito imediato e latente da temperatura do grão durante o processo de pulverização sobre a persistência e eficácia biológica do inseticida pirimifós-metil no controle de *Sitophilus zeamais* e *Tribolium castaneum*. Para tal, pulverizou-se o inseticida pirimifós-metil sobre os grãos de milho quando se apresentavam nas temperaturas de 25, 30, 35, 40 e 45 °C. A análise de resíduo foi realizada após a pulverização e a cada 30 dias, até 90 dias. Para avaliação da eficácia biológica 20 insetos adultos de *S. zeamais* e de *T. castaneum* foram colocados em uma placa de Petri contendo grãos tratados e, após 48 h de exposição dos insetos aos grãos, logo depois da pulverização e a cada 15 dias, até 90 dias, foram realizadas as avaliações. Observou-se que a eficácia biológica do pirimifós-metil reduziu durante o período de armazenamento e que o aumento da temperatura do grão no momento da pulverização também contribui para a redução da eficácia deste inseticida. Observou-se, também, que *S. zeamais* apresentou menor sensibilidade ao pirimifós-metil que *T. castaneum*.

Palavras-chave: *Sitophilus zeamais, Tribolium castaneum*, organofosforado, resíduo, controle de inseto

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INTRODUCTION

Several factors may contribute to the degradation of insecticides used for controlling insect pests in stored grains. Among them, air and grain temperature and moisture content are better studied and of major importance (Arthur et al., 1992; Wintersteen & Foster, 1992; White & Leesch, 1996; Fleurat-Lessard et al., 1998; Hamacher et al., 2002; Pimentel et al., 2004). High grain temperatures, for instance, cause fast breakdown of many insecticides, mainly by stimulating grain hydrolytic enzymes (Rowlands, 1975; Orth & Minett, 1975).

Dryeration techniques are increasingly used in Brazil and may impose a further problem for insect control with protectants in the tropics – the high grain temperatures during insecticide spraying. These techniques aim at energy savings, high drying capacity and lower thermal damage by long grain exposure to high temperatures (Silveira, 2002). If using dryeration, the warm grain is removed from the drier with up to 2.5% higher moisture content than recommended for storage. The grain is then transferred to a bin where it remains from 4 to 10 h to allow moisture distribution within the grain by residual heat. This process facilitates the removal of excess moisture during about 12 h of aeration, which will take place afterwards until the grain reaches the desired moisture content. However, the grain usually remains in the same bin after the resting period for the aeration, and the insecticide spraying takes place while conveying the grain from the drier to the bin, when grain temperature reaches over 45 °C (Mourier & Poulsen, 2000).

The organophosphate pirimiphos-methyl, one of the main grain protectants used against stored product insects in Brazil, is frequently used in these operations (Andrei, 1999) and the procedure described above (spraying at high grain temperatures) is a potential problem for stored product protection because organophosphate degradation is usually faster at high temperatures (Arthur et al., 1992; White & Leesch, 1996; Daglish, 1998; Fleurat-Lessard et al., 1998; Faroni et al., 2002; Hamacher et al., 2002; Pimentel et al., 2004) compromising even further the already difficult control of stored product insects in tropical areas. Therefore, the objective of the present study was to assess the impact of high grain temperatures during spraying on pirimiphos-methyl persistence and activity towards two important pest species of stored maize in the tropics – the maize weevil and the red flour beetle.

MATERIAL AND METHODS

The investigation was carried out in the Viçosa County, State of Minas Gerais, from December 2000 to January 2002, where maize grains of the variety AG-1051 harvested with 20% m.c. were dried to 15% m.c. in batches of 20 kg using an experimental drier with 1 m s^{-1} of drying air speed. Dried grains (15% m.c.) at different temperatures (25, 30, 35, 40 and 45 °C) were placed in a conveyor belt 1.0 m long and 0.2 m wide (Faroni et al., 2002; Hamacher et al., 2002). The belt capacity was 8.25 t ha^{-1} activated by an induction triphasic 0.5 hp motor coupled with a 0.5 hp motoreductor (with 24 x reduction). A container with flow regulation was coupled to the conveyor belt to guarantee uniform grain flow in a thin layer throughout its length. The sprayer used for insecticide spraying at the conveyor belt was equipped with a single flat fan nozzle (Teejet TP650067) at 0.15 m high and regulated to deliver an application rate of 11.5 mL min^{-1} at 2 bar pressure.

The organophosphate insecticide pirimiphos-methyl (Actellic® 500EC) was applied at the recommended concentration of 4.0 mg a.i. mL^{-1} and 1.5 mL of insecticide solution was used per kg of maize (= 1.5 L per tonne of maize). The grain batches were left to rest for 6 h after spraying and then they were aerated for 12 h until reaching 13% m.c. Four batches of maize grains were sprayed at each temperature and 2.0 kg samples were taken from each batch and stored at 27 ± 1 °C and 56 ± 5% r.h. until the residue analysis and bioassay tests. Grain samples sprayed with water only were also used for the residue analysis and bioassay tests. The tests were carried out at the same environmental conditions of storage.

Insecticide residues on grain samples sprayed at the different temperatures were analyzed 0, 30, 60 and 90 days after spraying using three replicates. Non-sprayed grain samples were also analyzed for pirimiphos-methyl residues at the same storage intervals. Only analytical standards of the solvents were used for the residue analysis. Technical grade pirimiphos-methyl (91.5%) was obtained from Zeneca Brasil (Holambra, SP, Brazil). The extraction methodology was adapted from Hamacher et al. (2002) and Luke et al. (1975). The efficiency of this extraction technique was assessed in three maize samples fortified with 1.0 mL standard solution of pirimiphos-methyl at 100.0 mg mL^{-1}, allowing 86.1% recovery of the active ingredient. Ten grains of maize from each sample where mixed with acetone (25 mL), hexane (10 mL) and dichlorometane (15 mL) in an Erhlenmeyer flask and shaken for 30 min. The extract was filtered through filter paper with 20 g of sodium sulfate and 15 mL dichlorometane was used to wash the paper at the end of the filtration. The extract was concentrated in a rotary evaporator at 40 °C and recovered to 4.0 mL with hexane. Sample extracts were analyzed in a gas chromatograph (Shymadzu CG-17A, Kyoto, Japan) equipped with a BP-5 column (poly – 5% diphenyl/95% dimethylsiloxane, 30 m X 0.25 mm i.d. and film thickness of 1 mm) and a flame ionization detector (FID) (Faroni et al., 2002; Hamacher et al., 2002). Temperature of the column was 220 °C (7 min; isothermic), while the injector was maintained at 250 °C and the FID detector was maintained at 300 °C. The flow of the carrier gas (N) was 1.2 mL min^{-1}, the split ratio was 1:5 and the injected volume was 1 mL. Under these conditions, the pirimiphos-methyl retention time was about 4 min. The setup values for the hydrogen, air and nitrogen make-up gas for the FID were 60 kPa (approx. 50 mL min^{-1}), 50 kPa (approx. 500 mL min^{-1}) and 75 kPa (approx. 30 mL min^{-1}), respectively. The residues were quantified directly from the calibration curve established within the detector linearity range, by comparing the sample peak areas with the peak areas of the external standards. The detector showed reasonable linearity within the work range from 0.2 to 10.0 mg mL^{-1}, with R^2 = 0.994, detection threshold of 0.2 mg mL^{-1} and quantification threshold of 0.6 mg mL^{-1}.

The biological activity of pirimiphos-methyl sprayed at the five different temperatures was independently evaluated using non-sexed adults of maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and of red flour beetle, *Tribolium castaneum* (Herst) (Coleoptera: Tenebrionidae). These insects were from laboratory colonies maintained at 27 °C and 55% r.h. on non-treated maize. Four 50 g samples of treated maize grains were taken from each treatment every 15 days up to 90 days after the insecticide spraying. These grain samples were placed in Petri dishes and infested with 20 insects of either species. They were maintained at 27 °C and 55% r.h. for 48 h, after which the insect mortality was assessed. Mortality was corrected for the natural mortality on samples sprayed with water only.

Multiple regression analysis (SAS Institute, 1997) were used to determine whether the different grain temperatures at spraying and storage periods affected residue levels of pirimiphos-methyl on maize grains and the biological activity of this insecticide towards the maize weevil and the red flour beetle. Non-linear regression analyses were used to establish the relationship between residue levels and biological activity of pirimiphos-methyl. These analyses were carried out using the curve fitting procedure of SigmaPlot (SPSS INC, 2000).

RESULTS AND DISCUSSION

The residue level of pirimiphos-methyl on the grains shortly after spraying (2 h) were significantly smaller when this operation was carried out at higher grain temperatures with a nearly 80% difference between the extreme temperatures (2.5 ± 0.0 ppm at 45 °C and 4.3 ± 0.4 ppm at 25 °C) (Figure 1). The differences remained, with time getting even greater at the end of the storage period, 90 days after spraying (0.2 ± 0.0 ppm at 45 °C and 0.5 ± 0.1 ppm at 25 °C). The decrease in residue level was inversely related to grain temperatures and storage time. The effect of the air temperature favoring degradation of organophosphate insecticide residues on stored grains is widely recognized (Arthur et al., 1992; White & Leesch, 1996; Fleurat-Lessard et al., 1998; Afridi et al., 2001).

High air temperatures, even if only during spraying, also showed a drastic effect on reducing pirimiphos-methyl residues on the grain surface and compromising insect control as demonstrated by Faroni et al. (2002) and Pimentel et al. (2004). In contrast, the effect of grain temperature in insecticide degradation was seldom explored. Wintersteen & Foster (1992) did not observe any significant effect of grain dried under different systems in malathion degradation, unlike what was reported for pirimiphos-methyl. Such differences may be due to structural differences between

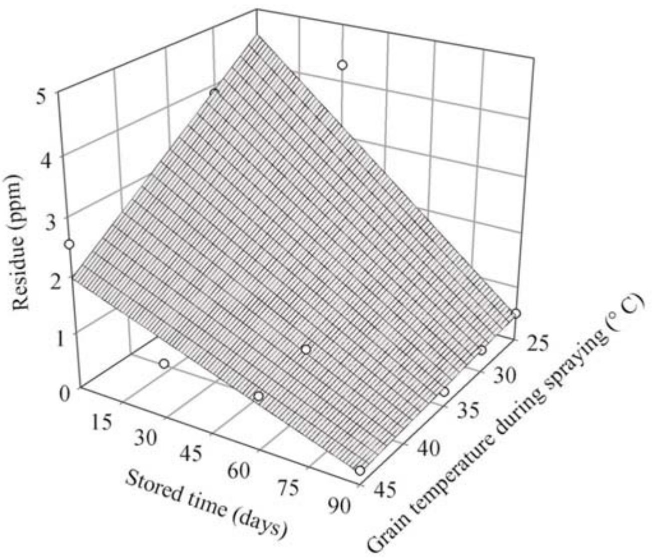

Figure 1. Effect of grain temperature, during spraying, and storage period (days) on the residue level of pirimiphos-methyl on maize grains. (y = 7.81 – 0.08x – 0.13z + 0.001xz; where y = residue level (ppm), x = storage period (days), and z = grain temperature during spraying (°C); R^2 = 0.67; F = 24.26; p < 0.0001; df_{error} = 31)

both compounds, but the high storage temperature used in the present study (27 °C) was a likely contributor to maximize insecticide degradation. The effect of the grain temperature during insecticide spraying on the degradation and activity of pirimiphos-methyl, demonstrated in our study, was expected based on previous studies assessing the effect of temperature speeding up insecticide degradation (Arthur et al., 1992; Daglish, 1998; Fleurat-Lessard et al., 1998; Afridi et al., 2001; Hamacher et al., 2002; Pimentel et al., 2004). However, residue levels of 50 to 80% higher at 25 than at 45 ºC immediately after spraying, with 2- to 4-fold drop during the 90-day storage period, were a surprise. The high grain temperatures at spraying may have led to insecticide losses through its suspension in the warm air surrounding the warm grain. This may have prevented the insecticide from reaching the grain surface and probably favored degradation on the grain surface as well. Evaporation of a volatile carrier (water in our case) can result in a decrease in droplet size to the point at which small droplets solidify, remaining suspended in the air and failing to adhere to the grain surface (Johnstone, 1985).

The decrease in the residue levels of pirimiphos-methyl in stored maize was also inversely related to mortality of the maize weevil and the red flour beetle (Figure 2). Insect mortality dropped quickly below 2 ppm, going from nearly 100% mortality to 0% for both insect species. Higher residue levels had negligible impact on insect mortality, which was already close to 100%. High temperatures generally result in fast insecticide degradation, which seems particularly true in the case of organophosphates (Arthur et al., 1992; White & Leesch, 1996; Daglish, 1998; Fleurat-Lessard et al., 1998). The lower persistence of pirimiphos-methyl residues on maize grains was translated into lower efficiency in controlling infestations of the maize weevil and red flour beetle. These relationships were non-linear, showing a great decrease in mortality with a decrease in residue levels from 2 to 1 ppm. Arthur et al. (1992) and Hamacher et al. (2002) also observed an increase in insect survival with a decrease in organophosphate residue levels on stored grains. The rate of reduction of residue levels indicates the length of protection provided by the insecticide. In all the instances investigated, with insecticide applications taking place with warm grains (from 25 to 45 °C) in a tropical area, the grain protection period will be greatly compromised.

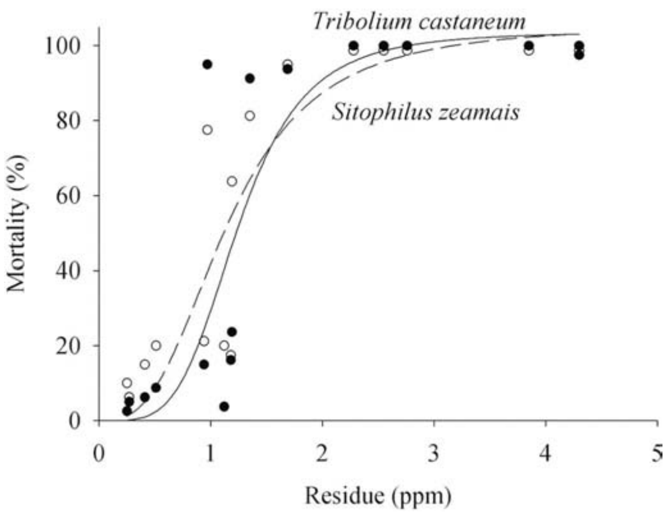

Figure 2. Relationship between residue levels of pirimiphos-methyl on stored maize and mortality of *Sitophilus zeamais* (open triangule; dotted line) and *Tribolium castaneum* (open circle; solid line). (*S. zeamais*: $y = 105.54/(1 + (x/1.71)^{-6.95}$, $R^2 = 0.80$, $F = 32.35$, $p < 0.0001$, $df_{error} = 14$; *T. castaneum*: $y = 103.68/(1 + (x/1.25)^{-4.16}$, $R^2 = 0.72$, $F = 21.58$, $p < 0.0001$, $df_{error} = 14$)

Insect mortality, as expected, decreased with an increase in grain temperature during spraying and period of storage (Figures 3A and 3B). Mortality was highest right after insecticide application for the highest temperature under investigation (i.e., 45 °C). The decrease in mortality was uniform with storage time and reached the lowest mortality levels faster when grains were sprayed at the highest temperatures for both insect species. There was no significant interaction between grain temperature at spraying and storage time for either insect species. A fast decrease was observed in residue levels and biological activity of pirimiphos-methyl during a 90-day storage period that was accentuated by the high grain temperatures during insecticide spraying. This finding is of importance because organophosphate spraying in tropical areas is frequently carried out on warm grain subjected to dryeration, which favors organophosphate degradation. A solution to minimize this problem is to avoid using the same bin for resting (allowing moisture distribution in the grain) and aeration (completing the grain drying). The use of two bins, one for resting the grain and another for aerating it, will allow spraying of the grain after its cooling at the resting bin while transporting it by the conveyor belt to the aerating bin. Such procedure will favor the residual effect of the insecticide providing suitable protection against insect-pests for longer storage periods.

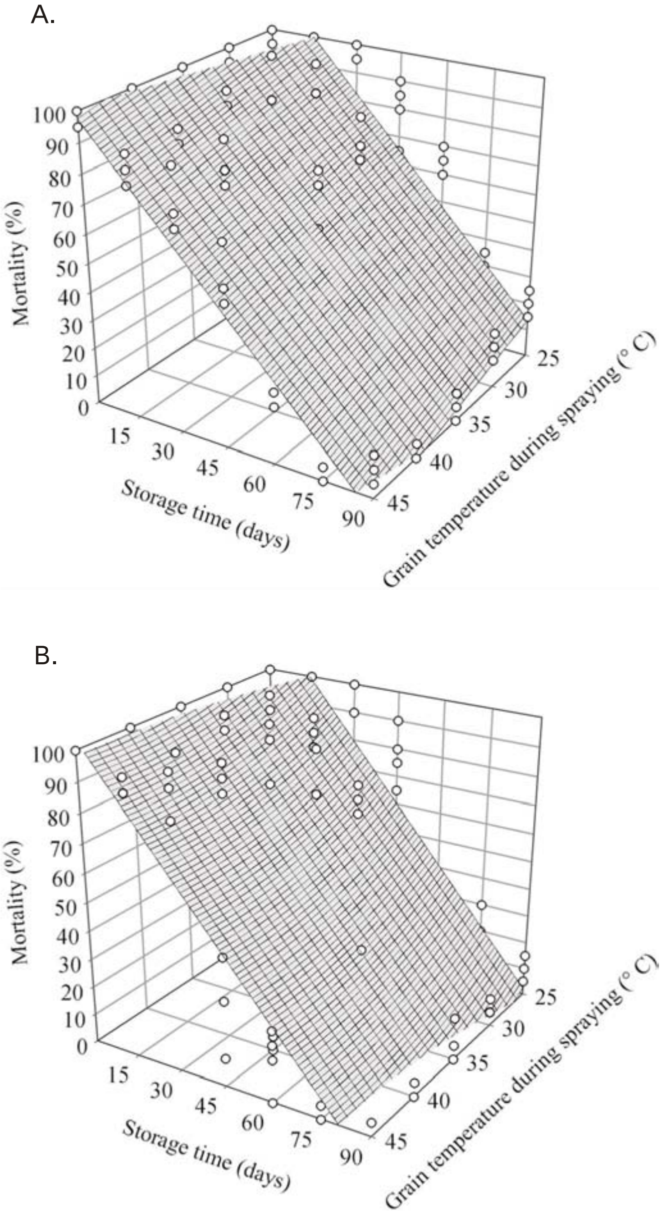

Figure 3. Effect of grain temperature during spraying with pirimiphos-methyl, and storage period (days) on the mortaltity of *Sitophilus zeamais* (A) ($y = 141.65 - 1.15x - 0.98z$, $R^2 = 0.87$, $F = 116.96$, $p < 0.0001$, $df_{error} = 32$) and *Tribolium castaneum* (B) ($y = 188.32 - 1.29x - 3.95z + 0.05z^2$, $R^2 = 0.84$, $F = 62.91$, $p < 0.0001$, $df_{error} = 31$)

CONCLUSION

These results clearly indicate the drastic effect of grain temperatures during spraying compromising the efficiency of grain protectants for insect pest control on stored grains.

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