CROP PROTECTION

Insecticide Selectivity to the Parasitic Mite *Acarophenax lacunatus* (Cross & Krantz) (Prostigmata: Acarophenacidae) on *Rhyzopertha dominica* (Fabr.) (Coleoptera: Bostrichidae)

José R. Gonçalves[1], Lêda R.D'A. Faroni[2], Raul N.C. Guedes[1] and Carlos R.F. de Oliveira[1]

[1]*Depto. Biologia Animal;* [2]*Depto. Engenharia Agrícola. Universidade Federal de Viçosa, 36571-000, Viçosa, MG*
e-mail: goncalves_mip@hotmail.com

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Seletividade de Inseticidas Para o Ácaro Parasita *Acarophenax lacunatus* (Cross & Krantz) (Prostigmata: Acarophenacidae) em *Rhyzopertha dominica* (Fabr.) (Coleoptera: Bostrichidae)

RESUMO - Diante da presença de inimigos naturais em massas de grãos armazenados e a necessidade de se buscar alternativas de controle de pragas, avaliou-se o efeito de inseticidas sobre o ácaro *Acarophenax lacunatus* (Cross & Krantz), um parasita de ovos de *Rhyzopertha dominica* (Fabr.). Os inseticidas bifentrina, deltametrina, pirimifós metílico e fenitrotiom foram testados a 0,4; 0,5; 0,8 e 10 ppm. As unidades experimentais consistiram de placas de Petri contendo grãos de trigo, infestados com 25 adultos de *R. dominica*. Sete dias após a infestação, foram inoculados três ácaros em cada placa, sendo utilizadas quatro repetições por tratamento. O número de adultos de *R. dominica*, a perda de matéria seca dos grãos e a taxa instantânea de crescimento (r_i) de *A. lacunatus* foram menores no tratamento com deltametrina. Os inseticidas bifentrina, fenitrotiom e pirimifós metílico apresentaram baixa eficácia na redução de *R. dominica*, mas foram seletivos em favor do ácaro, mostrando maior número de fêmeas fisogástricas e r_i para *A. lacunatus.* Com isso, conclui-se que deltametrina é o inseticida menos seletivo em favor de *A. lacunatus*. Entretanto, esse ácaro é capaz de parasitar ovos de *R. dominica* sob grãos tratados com todos os inseticidas avaliados.

PALAVRAS-CHAVE: Praga de grãos armazenados, ácaro, piretróide, organofosforado, controle biológico

ABSTRACT - The presence of natural enemies in stored grains and the need of pest control alternatives led to the assessment of the effect of insecticides on the mite species *Acarophenax lacunatus* (Cross & Krantz), an egg parasite of the stored grain pest *Rhyzopertha dominica* (Fabr.). The insecticides bifenthrin, deltamethrin, pirimiphos-methyl and fenitrothion were tested at 0.4, 0.5, 0.8 and 10 ppm, respectively. Insecticide treated wheat grains were spread in petri dishes and infested with 25 adults of *R. dominica.* Seven days later, three mites were added to each dish, in four replicates. The number of adults of *R. dominica*, grain weight loss and instantaneous rate of increase (r_i) of *A. lacunatus* were lower when the grain was treated with deltamethrin. Bifenthrin, fenitrothion, and pirimiphos-methyl were less effective in controlling the pest, but they were selective in favor of the mite, showed higher number of physogastric females and r_i of *A. lacunatus*. Therefore, deltamethrin was less selective in favor of the mite species. Nonetheless the parasitic mite was able to parasitize eggs of *R. dominica* on wheat treated with all the insecticides evaluated.

KEY WORDS: Stored grain pest, mite, pyrethroid, organophosphate, biological control

Insecticide use is considered the only control option when a pest population in stored grains reaches the economic threshold (White & Leesch 1996). However, due to the development of resistance in many pests to the majority of the insecticides (organophosphates and pyrethroids) commonly used for their control (Collins *et al*. 1993, Guedes *et al*. 1996, Lorini & Galley 1999), insecticide use is becoming increasingly unviable (Arthur 1996). Because few insecticides are registered for controlling stored grain pests and the registration of new products requires a long time and at a high cost (White & Leesch 1996), alternative methods such as integration of natural enemies and insecticides need to be developed (King & Nordlund 1992).

The majority of mites infesting stored grains are pests, but some of them are natural enemies of stored grain-pests (Cross & Krantz 1964). Interest in the efficacy and applicability of mites for integrated pest management systems has been increasing. Mites are small, have a short life cycle, and high reproductive potential (Scholler *et al*. 1997, Faroni *et al*. 2001). Integrating the use of predatory/parasitic mites and insecticides for stored-grain pest management requires knowledge of the impact of the insecticides on the natural enemies or, in other words, their selectivity to natural enemies (Baker & Arbogast 1995, Gonçalves *et al*. 2002).

The egg parasite *Acarophenax lacunatus* (Cross & Krantz) is studied aiming its use as biocontrol agent of stored-grain pests. The whitish-colored small (0.14 mm x 0.09 mm) adults have a phoretic behavior (uses the pests to spread in the grain mass). This mite develops over a wide temperature range, from 18°C to 41°C, with an optimum at about 30°C. The female adults are responsible for egg parasitism. They suck the egg contents and distend to a bright, spherical form. The body size increases by about five times, which characterizes progeny development (physogastry). The mite progeny emerge as adults ready to parasitize new host eggs. The mite *A. lacunatus* significantly reduces the population of *Rhyzopertha dominica* (Fabr.) (Faroni *et al*. 2000), which is an important pest of stored products (Rees 1996). The following study was carried out to assess the insecticide selectivity to *A. lacunatus* and to determine the possibility of integrating the use of this mite species with some insecticides commonly used for stored-grain pest control.

Material and Methods

Mass Rearing of *R. dominica*. *R. dominica* was reared on non-treated whole wheat grain in petri dishes in an environmental chamber at 30 ± 1°C, 65 ± 5% relative humidity (RH) and 24h scotophase. The insects originated from a laboratory colony maintained at the same conditions for four consecutive years without insecticide exposure. Each petri dish containing the whole wheat was infested with 50 adults of *R. dominica* and seven days later the eggs were collected by sieving through an 1-mm aperture sieve. The collected eggs were placed on the fresh diet in petri dishes. This period was sufficient for the pest to lay its eggs. Eggs of a known age were periodically collected in a similar way (Faroni *et al*. 2000).

Mass Rearing of *A. lacunatus*. Individuals of *A. lacunatus* were obtained from cultures of *R. dominica* infested with this parasite for more than four years. The mites were reared on the *R. dominica* at 30 ± 1°C, 65 ± 5% RH, but in a different room from uninfested insects (Faroni *et al*. 2000).

Insecticide Application. The insecticides used were bifenthrin (Prostore 25 CE), deltamethrin (K-Obiol 25 CE), pirimiphos-methyl (Actellic 500 CE) and fenitrothion (Sumigran 500 CE) applied at the concentrations of 0.4, 0.5, 8.0 and 10.0 ppm, respectively (doses within the range recommended by the manufacturers and commonly used in wheat stores). The experimental procedure followed the general guidelines standardized by the International Organization for Biological Control/West Paleartic Regional Section (IOBC/WPRS) for laboratory tests on the susceptible stages of arthropods (Hassan *et al*. 1994). Thus, the insecticides were tested at recommended rates using one-day old mites. The insecticide was dissolved in distilled water and, using a manual atomizer with the spray-nozzle turned downwards, 1 ml of solution was sprayed from a height of 20 cm on 1 kg of wheat grains within a wind protected container similar to a spray tower. To avoid cross-contamination among the insecticides, separate atomizers were used for each treatment. The control was sprayed with the same amount of distilled water. The atomizer was calibrated in a preliminary test, on 10 kg of wheat spread over a 1 m^2 surface area, receiving 1 ml of the solution for each kg of the grain.

Each experimental unit encompassed a 140 mm x 10 mm petri dish (diameter x height) containing 25 g of treated or non-treated wheat grain. Grains in each petri dish were infested with 25 non-sexed, three- to seven-day old adults of *R. dominica* (Faroni *et al*. 2000). Seven days after infestation with the pest, which allowed a suitable period for oviposition, three non-physogastric females of *A. lacunatus* were added to each experimental unit, with four replicates. The dishes and their contents were wrapped in a PVC plastic film to avoid insect or mite escape and to prevent possible contamination with individuals of other species. Four holes were made with entomological pins for air exchange in the PVC film covering the petri dishes.

The petri dishes were stored for 23 days in environmental chambers at 30 ± 1°C, 65 ± 5% relative humidity (RH) and 24h scotophase. A second generation of the pest did not complete development during this test period. The wheat weight loss (%) was determined through the changes in the weight at the beginning and at the end of the experiment. The grain mass from each dish was sieved through a 1 mm aperture sieve to separate the grains from the insects and the powder containing the eggs of *R. dominica* and the mites. The number of live and dead adults of *R. dominica*, parasitized eggs and mites in the physogastric process were determined in each experimental unit using a stereomicroscope.

The instantaneous rates of increase of *A. lacunatus* (r_i) were calculated using the equation: $r_i = [\mathrm{Ln}(N_f/N_o)]/\Delta t$; where N_f = final number of live mites; N_o = initial number of live mites; Δt = time variation (duration of the experiment in days) (Walthall & Stark 1997). All data were subjected to analysis of variance and the means were compared using Duncan's multiple range test at 5% probability level whenever appropriate.

Results

The number of physogastric females of *A. lacunatus* was significantly different among insecticides with higher numbers for bifenthrin and fenitrothion ($df_{error} = 15$; $F = 4.84$; $P = 0.01$). Similar trend was observed for instantaneous rate of increase of *A. lacunatus* (r_i) ($df_{error} = 15$; $F = 49.91$; $P < 0.001$), with lower population growth of *A. lacunatus* when deltamethrin was used (Table 1).

Table 1. Effect of four insecticides applied in wheat grains in the number of physogastric females and instantaneous rates of increase of *A. lacunatus*, and egg parasitism (%) (mean ± SE) of *R. dominica* maintained at 30 ± 1°C, 65 ± 5% relative humidity (RH) and 24h scotophase.

Treatments	Number of physogastric females of *A. lacunatus*	Instantaneous rates of increase of *A. lacunatus* (r_i)	Parasitism (%) of host eggs
Control	76.8 ± 25.01 a	0.1350 ± 0.0126 a	100.0 ± 0.00 a (486) [1]
Bifenthrin	62.5 ± 10.31 a	0.1300 ± 0.0082 ab	91.6 ± 1.10 a (520)
Deltamethrin	1.5 ± 0.96 b	0.0025 ± 0.0075 c	90.6 ± 9.38 a (36)
Pirimiphos-Methyl	16.0 ± 16.00 b	0.1308 ± 0.0008 ab	25.0 ± 25.00 b (765)
Fenitrothion	36.0 ± 4.90 ab	0.1068 ± 0.0058 b	89.0 ± 11.04 a (225)

Means followed by the same letter within each column are not significantly different by Duncan's multiple range test at 5% probability.
[1](n) Real number of eggs of *R. dominica* parasitized by *A. lacunatus*

There were also significant differences in parasitism of *R. dominica* eggs with the different insecticides (df_{error} = 15; F = 5.61; P = 0.006). Pirimiphos-methyl drastically compromised egg parasitism, which was significantly different from the others insecticides that allowed parasitism levels over 89% (Table 1).

There was no significant interaction between insecticides and *A. lacunatus* in reducing the number of adults of *R. dominica* (df_{error} = 30; F = 0.77; P > 0.05) and the effect of the parasitic mite alone was also non-significant for this parameter (df_{error} = 30; F = 0.14; P > 0.05). Only insecticides affected significantly the number of adults of *R. dominica* (df_{error} = 30; F = 90.25; P < 0.001).

The same trend was observed for wheat weight loss (%), where the insecticide-mite interaction (df_{error} = 30; F = 1.33; P = 0.28) and the mite effect (df_{error} = 30; F = 0.003; P > 0.05) were not significant. Deltamethrin showed the greatest reduction of adults of *R. dominica* and consequently the lowest wheat weight loss (Table 2).

The number of eggs of *R. dominica* was affected not only by the insecticide applied (df_{error} = 30; F = 5.46; P = 0.002), but also by the mite presence (df_{error} = 30; F = 4.45; P = 0.04). Nonetheless, the interaction between insecticides and mite presence was not significant (df_{error} = 30; F = 0.71; P > 0.05). The lowest numbers of *R. dominica* eggs were observed with deltamethrin use and the mite presence also significantly reduced the number of eggs of *R. dominica* (Table 3).

Table 2. Effect of four insecticides on wheat grains in the reduction of the number of *R. dominica* adults and wheat weight loss (mean ± SE) after 30 days of the insect-pest infestation, maintained at 30 ± 1°C, 65 ± 5% relative humidity (RH) and 24h scotophase.

Treatments	Reduction (%) of the number of adults of *R. dominica*	Wheat weight loss (%)
Control	5.0 ± 1.46 c	7.1 ± 0.28 a
Bifenthrin	21.0 ± 2.59 b	3.9 ± 0.17 c
Deltamethrin	73.0 ± 4.12 a	2.0 ± 0.22 d
Pirimiphos-Methyl	4.5 ± 1.76 c	4.1 ± 0.20 bc
Fenitrothion	12.0 ± 3.78 c	4.5 ± 0.17 b

Means followed by the same letter within each column are not significantly different by Duncan's multiple range test at 5% probability.

Discussion

The insecticides affected the number of physogastric females of *A. lacunatus*, with the greatest impact occurring with deltamethrin, followed by fenitrothion and pirimiphos-methyl. The number of *A. lacunatus* females was higher in grains treated with bifenthrin and fenitrothion indicating the selectivity of these insecticides in favor of the mite *A. lacunatus*. Similar results were reported for predatory mites with other insecticides and acaricides used in the management of stored product pests (Zdarkova & Horak 1984) and in the field (Croft *et al*. 1982). Zdarkova & Horak (1984) observed that none of the eight acaricides evaluated by them caused 100% mortality of the predatory mite *Cheyletus eruditus* (Schrank).

The instantaneous rates of increase of *A. lacunatus* (r_i) was low in the presence of deltamethrin, which may be either because of the non-selectivity of this insecticide or because of low numbers of host eggs that resulted from the reduced population of *R. dominica*. Similar results were found for the predators *Androlaelaps casalis* (Berl.), *Blattisocius keegani* Fox and *C. eruditus*, whose numbers were significantly reduced by chlorpyriphos-methyl at 6 ppm on stored oats (White & Sinha 1990).

Many of the insecticides used for pest control can be detrimental to populations of natural enemies (Baker *et al*. 1995). However, some insecticides that have low negative impact on the biocontrol agent can be successfully used to control stored-grain pests (White & Leesch 1996). A similar strategy can be applied if insecticide resistant lines of these biocontrol agents are available (Baker & Arbogast 1995, Baker *et al*. 1995), thus allowing an integrated pest management

Table 3. Effect of four insecticides on wheat grains in the number of eggs of *R. dominica* after 30 days the infested of those grains, maintained for 30 ± 1 ºC, 65 ± 5% relative humidity (RH) and 24h of scotophase.

Treatments	Number of eggs of *R. dominica*		Mean
	With mites	Without mites	
Control	121.5 ± 36.50	232.8 ± 41.10	177.1 ± 33.00 ab (1417)[1]
Bifenthrin	142.0 ± 21.30	133.0 ± 25.80	137.5 ± 15.60 b (1100)
Deltamethrin	9.8 ± 2.80	31.1 ± 12.00	20.4 ± 7.00 c (163)
Pirimiphos-Methyl	216.3 ± 63.50	300.0 ± 97.90	258.1 ± 56.30 a (2065)
Fenitrothion	64.8 ± 14.50	204.5 ± 94.50	134.6 ± 51.50 b (1077)
Mean	110.9 ± 21.20 B (2217)	180.3 ± 33.30 A (3605)	

Means followed by the same lower case letter in the column and the same capital letter in a row are not significantly different by Duncan's multiple rage test at 5% probability.
[1](n) Total number of eggs of *R. dominica*

approach for stored grains (Zdarkova 1994).

The high levels of parasitism observed with the insecticides bifenthrin, deltamethrin and fenitrothion suggest the possibility of integrating *A. lacunatus* with these insecticides for controlling *R. dominica*. This integration of these two methods of insect-pest control in a storage environment is important, especially in cases where the pests have already acquired resistance to most insecticides used in this ecosystem (Arthur *et al*. 1988, Collins 1990, Collins *et al*. 1993).

The number of *R. dominica* adults and eggs and wheat weight loss caused by insect-pest infestation was lowest in deltamethrin-treated grains. The efficacy of bifenthrin, fenitrothion and pirimiphos-methyl against *R. dominica* was lower, which suggests its tolerance (or more likely, resistance) to these products. Several authors have reported stored-products insect resistance to organophosphates such as malathion, pirimiphos-methyl and chlorpyriphos-methyl (Zettler & Cuperus 1990, Guedes *et al*. 1996) and also to pyrethroids such as bioresmethrin, fenvalerate (Collins *et al*. 1993) and deltamethrin (Collins *et al*. 1993, Lorini & Galley 1999). Other pests of stored products such as *Acanthoscelides obtectus* (Say), *Cadra cautella* (Walker), *Oryzaephilus surinamensis* (L.), *Plodia interpunctella* (Hubner), *Sitophilus oryzae* (L), *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* (Herbst) also have developed resistance to organophosphates and pyrethroids (Arthur *et al*. 1988, Collins 1990, Collins *et al*. 1993).

To increase the feasibility of combining biological and chemical control agents, the selected or existing populations of natural enemies effective against stored-grain pests should have their tolerance to stored-grain protectants determined (Baker *et al*. 1995). Combining the two control methods is important because the indiscriminate use of insecticides can lead to serious problems, including pest resistance, which is even more likely in this case because there are few registered products for stored-product protection and the cost of registering a new insecticide is very high (Woodhead *et al*. 1990, Hagstrum & Flinn 1996).

The parasitic mite *A. lacunatus* is an important biological control agent because besides having showed tolerance to the insecticides and high rates of parasitism on *R. dominica* eggs, its presence inhibits oviposition by this insect-pest. Deltamethrin was the most effective insecticide for reducing *R. dominica* populations resulting in reduced grain loss and for maintaining the mite population at low but effective levels (90.6 ± 9.38% egg parasitism). Although the efficacy of bifenthrin, fenitrothion and pirimiphos-methyl was low in reducing *R. dominica* adult populations, it resulted in greater availability of the host eggs and allowed faster mite population growth. The mite population growth is not likely to compromise grain quality because these arthropods are easily removed from the grain mass in their regular cleaning process before processing (Scholler & Flinn 2000). Flinn & Hagstrum (2001) showed that augmentative releases of the *Theocolax elegans* wasp in wheat decreased in 89% the damage caused by *R. dominica* besides reducing in 92% the insect fragments in the final product (flour).

These results provide support for the possible use of a biological control agent for stored grain pest management even with insecticide use. However, additional studies are still necessary, extending the investigation to other common pests in the stored-grain ecosystem and determining the effect of these insecticides on the biology of *A. lacunatus* in association with these pests.

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