

Efficacy of layer treatment with methoprene for control of *Rhyzopertha dominica* (Coleoptera: Bostrychidae) on wheat, rice and maize

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Abstract

BACKGROUND: Insect growth regulators are promising alternatives to traditional pesticides in stored grain. The efficacy of the juvenile hormone analogue methoprene was evaluated as a layer treatment in a laboratory experiment for control of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) in wheat, rice and maize.

RESULTS: Adults of *R. dominica* were placed in vials containing 33, 26 and 29 g (to a depth of 6.5 cm) of wheat, rice and maize, respectively, that was entirely or partially treated with 1, 5 or 10 mg kg⁻¹ methoprene. In wheat and rice, the layer treatments were not as effective as the whole-grain treatment, but there was decreased progeny production as the application rate increased. However, on maize the partial treatments were as effective as the whole-grain treatment at 5 and 10 mg kg⁻¹.

CONCLUSIONS: The results suggest that partial layer treatments with methoprene can be used to control *R. dominica* on maize but may not be effective for control of this species on wheat and rice.

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Keywords: grain protectants; insect growth regulator; methoprene; *Rhyzopertha dominica*; stored-product insects

1 INTRODUCTION

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), is a cosmopolitan pest of stored grains. The adults feed on whole grains, and females lay eggs on the outside of kernels. Both adults, which can live for more than 6 months, and larvae are responsible for the grain infestation. The eggs hatch, the neonates bore into kernels and immatures develop inside the kernel. *Rhyzopertha dominica* is resistant to the fumigant phosphine^{1–3} and to some neurotoxic grain protectants.^{1,4–6} Insect growth regulators (IGRs) are alternatives to traditional neurotoxic contact insecticides.^{7,8} Juvenile hormone analogues (JHAs), such as fenoxycarb, methoprene and hydroxyphenoxycarb, do not normally affect adults but interfere with normal growth and development of immature insects and can also confer some egg toxicity.^{7,9}

IGRs, and JHAs in particular, are not toxic to mammals, can be applied to grain with the same technology as other protectants and have reduced application risks compared with neurotoxic insecticides. Methoprene is the only IGR registered as a grain protectant in the United States (USA) and has been shown to reduce progeny production of several stored-grain insect pests, including *R. dominica*.^{6,7,9–12} With many grain protectants, efficacy can vary depending on the specific grain commodity, but there is little published research concerning the activity of methoprene on grains other than wheat.^{9,13} The application of an insecticide on the upper surface of the grain mass, known also as 'top dressing', is one of the solutions suggested in order to reduce the total amount of residues in the final product. However, the performance of such

an application is highly dependent on insect movement. In a recent study, Athanassiou *et al.*¹⁴ examined the efficacy of spinosad as a layer treatment in wheat, and they reported that *R. dominica* adults did not readily move upward (i.e. showed positive geotaxis). In addition, *R. dominica* adults were so susceptible to spinosad that a short contact time with the treated wheat produced complete mortality, even when the exposed adults were transferred to untreated wheat. Several studies indicate that an initial exposure to methoprene by parental adults resulted in reduced fecundity of those adults.^{11,15} An application to only the surface layer of a grain mass could reduce the cost of insecticide treatment and also result in reduced residues in the grain mass. The objectives of the present study were: (1) to evaluate partial grain treatments with methoprene to control *R. dominica*, using several application rates under laboratory conditions in small grain columns; (2) to determine whether efficacy varied among wheat, rice and maize.

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Table 1. Dunnett's estimates for comparisons of progeny numbers in each of the rate–layer–grain combinations with progeny numbers in controls (in all cases, Dunnett's $d = 2.8$, $\alpha = 0.05$)

Treatment compared with control	Wheat		Rice		Maize	
	d	P	d	P	d	P
One-eighth treated with 1 mg kg ⁻¹	-80.2	0.99	-36.8	0.99	2.4	<0.01
One-eighth treated with 5 mg kg ⁻¹	-69.1	0.98	3.4	0.03	7.1	<0.01
One-eighth treated with 10 mg kg ⁻¹	-80.7	0.99	3.6	0.03	7.0	<0.01
One-fourth treated with 1 mg kg ⁻¹	-84.5	0.99	13.7	<0.01	4.4	<0.01
One-fourth treated with 5 mg kg ⁻¹	-83.6	0.99	-1.82	0.06	9.6	<0.01
One-fourth treated with 10 mg kg ⁻¹	-76.8	0.99	14.0	<0.01	10.2	<0.01
One-half treated with 1 mg kg ⁻¹	-54.8	0.82	18.0	<0.01	2.8	<0.01
One-half treated with 5 mg kg ⁻¹	-46.4	0.65	17.3	<0.01	8.6	<0.01
One-half treated with 10 mg kg ⁻¹	-16.7	0.15	32.6	<0.01	9.4	<0.01
All grain treated with 1 mg kg ⁻¹	13.5	0.02	44.1	<0.01	10.4	<0.01
All grain treated with 5 mg kg ⁻¹	13.7	0.02	44.9	<0.01	10.3	<0.01
All grain treated with 10 mg kg ⁻¹	13.6	0.02	44.6	<0.01	9.7	<0.01

2 MATERIALS AND METHODS

Rhyzopertha dominica were reared on whole wheat in laboratory cultures at 27 °C, 65% RH and continual darkness. Adult beetles (less than 3 weeks old) were used in the tests. The methoprene formulation used was an EC containing 336 g L⁻¹ (S)-methoprene, Diacon II (Wellmark International, Schaumburg, IL). Untreated, clean and infestation-free wheat, *Triticum aestivum* L. (a mixture of the var. Fuller and Santa Fe), rice, *Oryza sativa* L. (var. Francis), and maize, *Zea mays* L. (hybrid Golden Harvest H-8713), were used in the experiments.

The moisture content of the each of the three grains was adjusted to 13.5% before the experiments were initiated. Three lots of each commodity, consisting of 2 kg each, were sprayed with methoprene at application rates of 1, 5 and 10 mg kg⁻¹, in accordance with the label specifications of 0.7 mL of formulated spray per kg of commodity. The label specifies treatment in a range of 1–5 mg kg⁻¹. A Badger 100 artist's airbrush (Badger Air-Brush Company, Franklin Park, IL) was used to spray the required amount of spray (1.4 mL) for each application rate on each commodity. The respective quantities for each spray were placed on a thin layer before the application, in order to achieve equal distribution of the insecticide in the entire grain mass. An additional series of 2 kg commodity lots was sprayed with water and used as untreated controls, or as untreated commodity below the treated layer (as explained below).

Plastic cylindrical vials (3 cm diameter, 8 cm height) were used as the experimental units. Each vial was filled with a specific quantity of wheat, rice or maize, which filled the bottom 6.5 cm of the vials. This height corresponded to approximately 33, 26 and 29 g of wheat, rice and corn respectively. For each commodity and rate, separate treatment categories of vials were prepared, with three vials for each treatment. These categories were: (1) vials containing untreated commodities; (2) vials containing commodities entirely treated with methoprene; (3) vials in which the upper half of the grain was treated with methoprene and the bottom half was untreated; (4) vials in which the upper one-fourth of the grain was treated with methoprene and the rest was untreated; (5) vials in which the upper one-eighth of the grain was treated with methoprene and the rest was untreated. This gave 39 vials in total for each commodity (three control vials and 12 treated or partially treated vials for each rate). After the introduction of the grain into

the vials, ten 1–2-week-old unsexed adult *R. dominica* were placed in each vial. The vials were placed in incubators set at 27.5 °C, 75% RH and continual darkness. The numbers of live adults in the vials was determined after 65 days, as recommended by Arthur.¹⁰ These numbers also included parental (initial) adults, given that parental adults were not removed from the vials. This entire experimental procedure was repeated twice more, as described above for each application rate on each commodity.

Because of the experimental design of controls consisting entirely of untreated grain, a separate one-way ANOVA was used to compare the numbers of adults in untreated wheat, rice and maize using the Tukey–Kramer HSD test at 0.05 to separate the means.¹⁶ Also, adult progeny in each grain–rate–layer combination were compared with adult progeny in the control vials using Dunnett's test at $\alpha = 0.05$. Insect numbers in the vials containing treated grain were analysed using a three-way ANOVA for grain, rate and layer as the main effects. Means were separated using the HSD test, as above.¹⁶

3 RESULTS

3.1 Adult numbers in the untreated grains, and comparison of treated with untreated grains

There were significant differences in progeny production among the three untreated commodities ($df = 2, 24$; $F = 5.8$; $P < 0.01$). The number of adults was greater in wheat (122.7 ± 32.0 adults vial⁻¹) and rice (100.2 ± 18.5 adults vial⁻¹) than in maize (23.9 ± 2.7 adults vial⁻¹). There were no significant differences in numbers of progeny produced between vials containing treated wheat and the control vials, except for the vials that contained wheat that was entirely treated (Table 1). However, for rice, most of the combinations tested differed significantly from the control. For maize, all treated combinations had significantly lower adult numbers than the control.

3.2 Adult numbers in the treated grains

Commodity and treated layer, and their interaction, were the only significant effects (Table 2). There were no significant differences among rates for wheat and rice within each layer (Tables 3 and 4). At each dose, the number of *R. dominica* adults generally decreased as the depth of the treated layer increased, and few progeny were

Table 2. Main effects and interactions for *Rhyzopertha dominica* counts (total df = 323)

Source	df	F	P
Commodity	2	68.3	<0.01
Layer	3	30.1	<0.01
Commodity × Layer	6	11.0	<0.01
Rate	2	1.9	0.14
Commodity × rate	4	0.3	0.89
Layer × rate	6	0.3	0.93
Commodity × layer × rate	12	0.5	0.88

found in totally treated wheat. About ten adults were found in vials where the entire grain mass was treated. In contrast, more than 100 adults vial^{-1} were found in wheat when the upper 1/8 or 1/4 of the grain was treated, and 40–80 adults were found when the upper half of the wheat was treated. When the top half of rice was treated, there were 21–37 adults present, and 40–91 adults were present when the upper 1/4 to 1/8 of rice was treated.

No more than 16 adults were found in any of the maize treatments (Table 5). Within each layer there were significant differences among doses, with the exception of vials containing totally treated maize. In each layer treatment, numbers of adults were significantly lower in the 5 and 10 mg kg^{-1} treatments than in the 1 mg kg^{-1} treatment. At 1 mg kg^{-1} , fewer adults were found in totally treated maize than in the other treatments. At 5 mg kg^{-1} , fewer adults were found in the totally treated maize than on maize with the top 1/8 treated. No significant differences in adult numbers were noted among treatments at 10 mg kg^{-1} .

4 DISCUSSION

In previous studies, treatment of an entire experimental unit of grain with 1 mg kg^{-1} methoprene gave effective control of *R. dominica*.^{6,9–11} Chanbang *et al.*⁹ showed nearly complete suppression of *R. dominica* progeny on rice treated with 1 mg kg^{-1} , while Samson *et al.*¹³ reported residual control of *R. dominica* progeny for 48 weeks on rice and maize at this same rate. The present results indicate that treatment of a part of the grain quantity with methoprene may reduce progeny production of *R. dominica*, at least at the laboratory level tested here.

Surtees¹⁷ reported that *R. dominica* adults exhibit a positive geotaxis (downward vertical movement) from the top of a grain mass. Thus, adults of this species can penetrate through the

treated layer to oviposit in the untreated wheat. Vardeman *et al.*¹⁸ described penetration of adults through a layer of wheat treated with diatomaceous earth (DE); however, the progeny production assessed in that test was a direct consequence of parental mortality after exposure to the DE. In the present test there should have been no parental mortality; hence, the number of progeny was directly related to the depth of the treated layer in the experimental unit. Consequently, if *R. dominica* can penetrate through a surface layer treated with methoprene, then they can oviposit in the untreated wheat. Based on the present results, treatment of the entire commodity with methoprene was always superior to the layer treatment, regardless of the layer depth and the methoprene rate. This could indicate ovipositional preference for untreated wheat, failure to move back up into the treated layer once the adults reached the bottom of the grain in the experimental units (the 8 cm tall vials) or avoidance of the treated layer. Generally, increasing the depth of the treated layer in wheat and rice resulted in decreased progeny production. Methoprene has been shown to affect egg hatch rates and female fecundity in *R. dominica*. Chanbang *et al.*⁹ noted that larvae develop from eggs exposed to methoprene and can bore into the kernels, but do not emerge as adults. In the same study, average egg production of newly emerged *R. dominica* females, after exposure on rice treated with 1 mg kg^{-1} of methoprene, was reduced to less than 25% in comparison with females from untreated rice. Daglish and Pulvirenti¹⁵ also reported reduced oviposition of *R. dominica* females that were previously exposed for 7 days on methoprene-treated wheat. Similar results have been published by Loschiavo¹⁹ for the red flour beetle, *Tribolium castaneum* (Herbst), and the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae).

In contrast, the present results show that methoprene was effective as a layer treatment on maize, although numbers of adults in all treatments only ranged from 8 to 16. Indirectly, this is partially related to the reduced developmental parameters of *R. dominica* on this commodity, in comparison with wheat or rice.^{13,20} Moreover, in the present study, adult progeny production on maize was 19 and 24% of the corresponding values for wheat and rice respectively, and the increasing application rate of methoprene caused a further decrease in progeny production in the layer treatments. At 10 mg kg^{-1} , progeny production was not affected by the depth of the treated layer, indicating some potential for layer treatments using methoprene on maize. However, treating the entire experimental unit of maize with methoprene eliminated progeny production. There was also an indication of parental mortality on maize that was not seen on wheat and rice. Chanbang *et al.*⁹ also noted an unexpected level of mortality of parental *R. dominica* adults exposed

Table 3. Number of live adults vial^{-1} [mean (\pm SE)] in wheat treated in varying proportions with different rates of methoprene, 65 days after the introduction of ten parental individuals (within each row, means are not significantly different; within each column, means followed by the same lower-case letter are not significantly different; HSD test at 0.05, between rows df = 2, 24, between columns df = 3, 32)

Part of grain treated	Methoprene rate (mg kg^{-1})			F	P
	1	5	10		
Top one-eighth	141.3 (\pm 35.6) a	152.4 (\pm 32.3) a	140.8 (\pm 44.4) a	0.1	0.97
Top one-fourth	137.0 (\pm 27.0) a	107.4 (\pm 29.8) ab	100.7 (\pm 23.5) a	0.5	0.60
Top half	78.7 (\pm 14.6) b	70.2 (\pm 11.4) b	40.6 (\pm 13.0) b	2.3	0.12
Entire mass	10.3 (\pm 0.3) c	10.1 (\pm 0.1) c	10.2 (\pm 0.1) c	0.3	0.73
F	6.8	7.0	5.1		
P	<0.01	<0.01	<0.01		

Table 4. Number of live adults vial^{-1} [mean (\pm SE)] in rice treated in varying proportions with different rates of methoprene, 65 days after the introduction of ten parental individuals (within each row, means are not significantly different; within each column, means followed by the same lower-case letter are not significantly different; HSD test at 0.05, between rows $df = 2, 24$, between columns $df = 3, 32$)

Part of grain treated	Methoprene rate (mg kg^{-1})			F	P
	1	5	10		
Top one-eighth	91.0 (± 21.4) a	50.8 (± 12.9) a	50.6 (± 16.0) a	1.8	0.18
Top one-fourth	40.4 (± 7.1) b	56.0 (± 12.5) a	40.2 (± 8.3) ab	0.9	0.42
Top half	36.2 (± 11.1) b	36.9 (± 8.2) a	21.6 (± 6.4) b	1.0	0.39
Entire mass	10.1 (± 0.1) c	9.3 (± 0.3) b	9.5 (± 0.2) c	2.6	0.09
F	7.2	4.4	3.4		
P	<0.01	0.01	0.02		

Table 5. Number of live adults vial^{-1} [mean (\pm SE)] in maize treated in varying proportions with different rates of methoprene, 65 days after the introduction of ten parental individuals (within each row, means followed by the same upper-case letter are not significantly different; within each column, means followed by the same lower-case letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at 0.05, between rows $df = 2, 24$, between columns $df = 3, 32$)

Part of grain treated	Methoprene rate (mg kg^{-1})			F	P
	1	5	10		
Top one-eighth	16.0 (± 1.2) Aa	12.0 (± 0.9) Ba	12.0 (± 1.6) B	4.4	0.02
Top one-fourth	14.7 (± 1.2) Aa	9.4 (± 0.6) Bb	8.8 (± 1.0) B	11.0	<0.01
Top half	16.2 (± 1.6) Aa	10.4 (± 0.5) Bab	9.7 (± 0.7) B	11.5	<0.01
Entire mass	8.7 (± 0.4) b	8.8 (± 0.4) b	9.3 (± 0.4)	0.8	0.47
F	9.5	5.0	1.9		
P	<0.01	<0.01	0.15		

on methoprene-treated rice. This level of parental mortality was higher in long-grain rice than in short-grain rice, suggesting that an interaction may have been present that affected adult survival.

In conclusion, methoprene applied as a layer treatment in the present small-scale tests was not effective as a control for *R. dominica* on wheat and rice, as assessed by progeny production, but few adult progeny were produced on maize. Nevertheless, it should be noted that the present data describe the efficacy of layer treatment with methoprene in small columns of 8 cm (vials), and these results may not be transferable for a wider application in storage facilities owing to the fact that both treated and untreated layers are considerably larger. However, layer treatments of methoprene may be effective when used on a larger scale, such as in a grain bin, where a 1/8 layer treatment may require an *R. dominica* to travel across a metre of treated grain at the top of the grain mass before reaching untreated grain below. Methoprene can persist in the grain mass and provide long-term protection against several major insect species.^{12,13,21} Hence, in spite of the absence of parental mortality, methoprene may gradually eliminate the insect population by reducing both progeny and female fecundity. A combination of methoprene with a neurotoxic insecticide may give more complete control of *R. dominica*⁶ by increasing the parental mortality. Additional experimentation is required to determine the efficacy of specific combinations used as a layered treatment in stored grains.

ACKNOWLEDGEMENTS

The authors thank Ann Redmon and Ngunza Kisangani for technical support. They also thank Wellmark International (Schaumburg, IL) for providing the methoprene sample, and Pat Collins for his

comments on an earlier version of this manuscript. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture or the University of Thessaly.

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