



## Efficacy of spinosad in layer-treated wheat against five stored-product insect species<sup>☆</sup>

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### ABSTRACT

The biological insecticide spinosad was evaluated in laboratory bioassays as a surface treatment for wheat to control adult *Rhyzopertha dominica*, *Sitophilus oryzae* and three psocid species, *Liposcelis paeta*, *L. bostrychophila*, and *Lepinotus reticulatus*. Spinosad was applied at 1 ppm to 35 g of wheat placed in a vial or to the upper one half, one fourth, or one eighth layer of the wheat; insects were either added to the vials before or after the wheat. When *R. dominica* were introduced into the vials after the wheat, mortality was 100% except for 83% mortality in the one eighth layer treatment. In contrast, when adults were placed in the vials before the wheat, mortality was 100% only when all of the wheat was treated. Mortality of *S. oryzae* was lower compared to *R. dominica* but there was some evidence of upward movement into the treated layers. Mortality of *L. paeta* and *L. bostrychophila* was <50% when the entire quantity was treated, in contrast to 100% mortality of *L. reticulatus*. However, for all psocid species, overall mortality decreased with decreasing depth of the treated layer. The results of this laboratory study show that while spinosad has some effectiveness as a layer treatment on a column of wheat, efficacy will be dependent on the target species, the depth of the treated layer, and the upward or downward mobility of the insect species.

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### 1. Introduction

The use of residual insecticidal protectants is a common preventative measure to protect stored grain from insect damage. Many of these insecticides are effective at relatively low dose rates and can provide long-term protection, which can range from 6 to 12 months (Arthur, 1996; Athanassiou et al., 2004). However, some grain protectants, particularly organophosphorus compounds (OPs), have high mammalian toxicity, and the residues left by these insecticides may cause health concerns because they are conventional neurotoxins that affect human nervous systems. This fact, combined with the development of resistance in many major stored-product insect species (Arthur, 1996), has resulted in the

development and evaluation of reduced-risk insecticides, which are more specific to insects and cause less environmental concerns to non-target organisms. One of these insecticides is spinosad, which is based on the metabolites of the bacterium *Saccharopolyspora spinosa* Mertz and Yao (Bacteria: Actinobacteridae). Spinosad is a broad-spectrum insecticide with low mammalian toxicity, and it is effective against many stored-grain insect species (Fang et al., 2002; Nayak et al., 2005). Moreover, spinosad gives excellent residual control, which makes it an ideal protectant for stored grain commodities (Fang et al., 2002; Fang and Subramanyam, 2003).

In grain bulks, high insect numbers are likely to be found only in some layers of the entire product mass. For instance, Weston and Barney (1998) found that in bins, where false floors exist, infestation can occur from the bottom of the bulk. Vela-Coiffier et al. (1997) noted that, for some species, immigration from the peripheral zones is very common, and can lead to increased infestations. On the other hand, several studies document that insect and mite densities are usually higher in the upper layer of grain bulks (Hagstrum, 1989; Hagstrum et al., 1998; Athanassiou et al., 2003). For instance, Athanassiou et al. (2003) reported that the majority of the insect and mite species found in a flat grain

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storage system were in the top 0.5 m of the bulk mass. For this last scenario, the application of contact insecticides to the upper layer of the grain bulk may cause a significant reduction in pest populations. Application only to the surface layer could reduce economic costs as well as the total amount of residues on the grain. Moreover, the use of a reduced-risk insecticide, such as spinosad, could also help alleviate concerns regarding the use of insecticidal protectants. Currently, there are no published reports in the scientific literature regarding efficacy of spinosad as a layer treatment to wheat. The purpose of our study was to determine if layer applications of spinosad would be an effective treatment for selected stored-grain pest species.

## 2. Materials and methods

### 2.1. Insects

The species evaluated in the experiments were the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and three psocid species: *Liposcelis paeta* Pearman, *L. bostrychophila* Badonnel (Psocoptera: Liposcelididae), and *Lepinotus reticulatus* Enderlein (Psocoptera: Trogiidae). The beetles were reared on whole wheat at 27 °C and 65% relative humidity (r.h.), while the psocids were reared on a mixture of cracked wheat, rice crispies, and brewer's yeast (at a ratio of 97:2:1) at 30 °C and 75% r.h. (Opit and Throne, 2008). Adult beetles <2 week-old were used in the tests, while adult female psocids <3 week-old were used, following the rearing techniques described by Opit and Throne (2008).

### 2.2. Commodity and insecticide treatment

Untreated whole wheat adjusted to 13.5% moisture content was used for the tests. The insecticide formulation was spinosad NAF 313 (13% active ingredient [AI]), obtained from Dow AgroSciences (Indianapolis, IN, USA). Separate formulations were prepared for each of three replicates and applied to a replicate lot of 2 kg of wheat by using a Badger 100 artist's airbrush (Badger Air-Brush Company, Franklin Park, IL, USA) to spray the wheat at the rate of 1 ppm, using a volume rate of 0.7 ml of formulated spray per kg of wheat, which is equivalent to the label rate for commercial grain protectants.

### 2.3. Bioassays

Plastic cylindrical vials (3 cm in diameter, 8 cm tall) were the experimental units for the test. Each vial was filled with 35 g of wheat, which covered the bottom of the vials to a depth of 6.5 cm. Thirty vials were prepared for each species as follows: (1) vials containing untreated wheat; (2) vials containing treated wheat; (3) vials in which the upper half of the grain was treated and the bottom half was untreated; (4) vials in which the upper one fourth of the grain was treated and the rest was untreated; and (5) vials in which the upper one eighth of the grain was treated and the rest was untreated. In half of the vials (15) the adults were placed in the vials before the introduction of the grains (the grain layers were poured on top after the introduction of the insects into the vials), while the adults were added after the introduction of the grains in the other half (the insects were introduced at the top after the introduction of the layers into the vials). Twenty adults were placed in each vial, with separate vials for each species. All vials were then placed at 27.5 °C, 75% r.h., and in continuous darkness. Mortality of the exposed individuals was assessed after 14 days. The same procedure was repeated three times (three series of vials) by preparing new vials each time.

### 2.4. Data analysis

Control mortality was low, so no correction for control mortality was considered necessary. The mortality counts were analyzed separately for each species by using a 2-way ANOVA with JMP software (Sall et al., 2001), with insect mortality as the response variable and treatment (depth of the treated layers) and method of insect introduction into the vials (before or after) as main effects. Means were separated by the Tukey–Kramer HSD test, at 0.05 probability (Sokal and Rohlf, 1995).

## 3. Results

Both main effects, treatment and method of introduction, and their interaction, were significant for *R. dominica* (Table 1). In the vials containing wheat totally treated with spinosad, all adults died whether added before or after the wheat (Fig. 1A). Moreover, when insects were introduced after the grain, mortality was 100% in all cases except vials containing wheat with just the top one eighth treated with spinosad, where mortality was 83%. In contrast, when *R. dominica* adults were placed in the vials before the grain, mortality was low when only a part of the grain was treated. Moreover, no significant differences ( $P \geq 0.05$ ) were noted in mortality levels among vials containing untreated wheat, regardless of the amount of wheat that was treated.

All main effects and interactions were significant ( $P < 0.05$ ) for *S. oryzae* (Table 1). In vials containing wheat totally treated with spinosad, significant differences were noted in adult mortality between the two systems of insect introduction (Fig. 1B). When weevils were put in the vials before the wheat, mortality did not exceed 78%, in contrast to 99% mortality when weevils were put into the vials after the wheat. When the upper one half of the grain was treated, the method of insect introduction was significant ( $P < 0.05$ ), but there was no difference when only one fourth of the grain was treated, with mortality not exceeding 47%. Finally, when one eighth of the wheat was treated, no significant differences were noted, and mortality was low (7 and 15%, for weevils introduced before and after the wheat, respectively).

Only treatment was significant ( $P < 0.05$ ) for *L. paeta* (Table 1), and mortality was lower compared to *R. dominica* and *S. oryzae* (Fig. 2A). Generally, adult mortality was higher in vials containing wheat totally treated with spinosad, in comparison with the vials containing treated and untreated quantities, but nonetheless mortality did not exceed 41%. As for *L. paeta*, only treatment was significant for *L. bostrychophila* (Table 1). Mortality was significantly higher in vials containing wheat totally treated with spinosad, as compared with the other treatments, while the decrease of the treated layer significantly decreased insect mortality, with total mortality of <47% for all treatments (Fig. 2B). Both main effects, as well as the interaction were significant for *L. reticulatus* (Table 1). All females were dead after 14 days on wheat totally treated with spinosad, regardless of whether the insects were added before or after the wheat (Fig. 2C). Also, no significant differences were noted in mortality levels in vials containing one half of treated wheat. Conversely, the timing of insect introduction into the vials was significant in the vials containing one fourth or one eighth treated wheat. Mortality of *L. reticulatus* increased with increasing depth of the treated layer only when they were placed in the vials before they were filled with wheat.

## 4. Discussion

Based on the results of the present study for wheat, the depth of the treated layer is an important factor impacting on the effectiveness of spinosad. For *R. dominica* and *S. oryzae*, insect placement

**Table 1**  
ANOVA parameters for main effects and interactions for the five species tested (total df = 71).

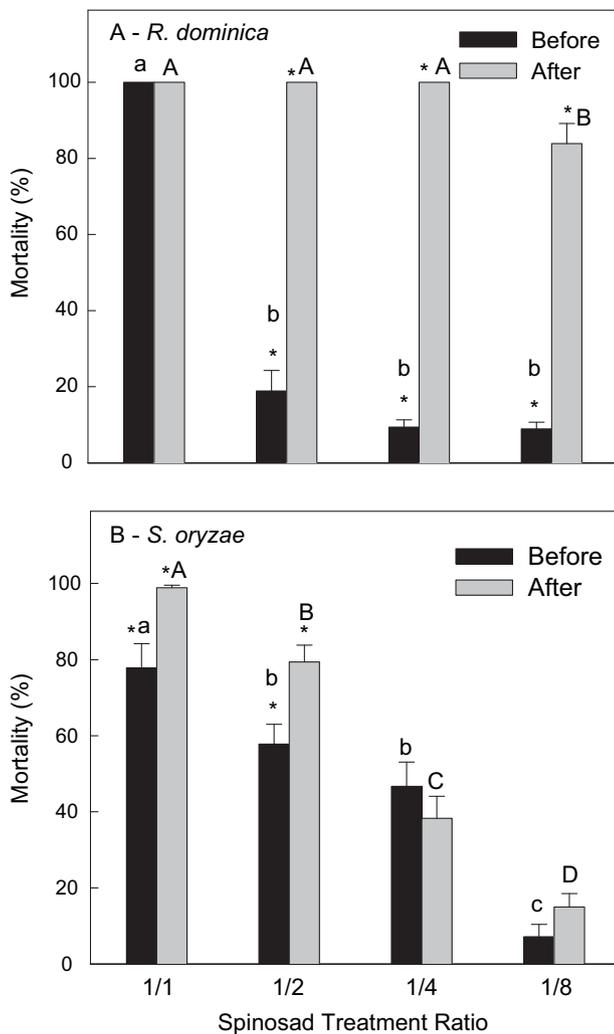
Source	df	Species									
		<i>R. dominica</i>		<i>S. oryzae</i>		<i>L. paeta</i>		<i>L. bostrychophila</i>		<i>L. reticulatus</i>	
		F	P	F	P	F	P	F	P	F	P
Insect placement	1	2048.1	<0.001	13.9	<0.001	0.3	0.554	0.1	0.921	18.6	<0.001
Treatment	3	306.6	<0.001	139.6	<0.001	19.9	<0.001	28.9	<0.001	63.5	<0.001
Placement × Treatment	3	233.1	<0.001	6.2	0.001	0.3	0.810	0.4	0.729	3.4	0.021

(before or after the grain) in the experimental vials was equally or more important than the depth of the treated layer. For the psocid species, the depth of the treated layer was the decisive factor for adult mortality. In a previous study with the organophosphate chlorpyrifos-methyl, partial treatment of maize at a ratio of 2:3 treated/untreated maize was equivalent to control with full treatment against the maize weevil, *Sitophilus zeamais* Motschulsky, and

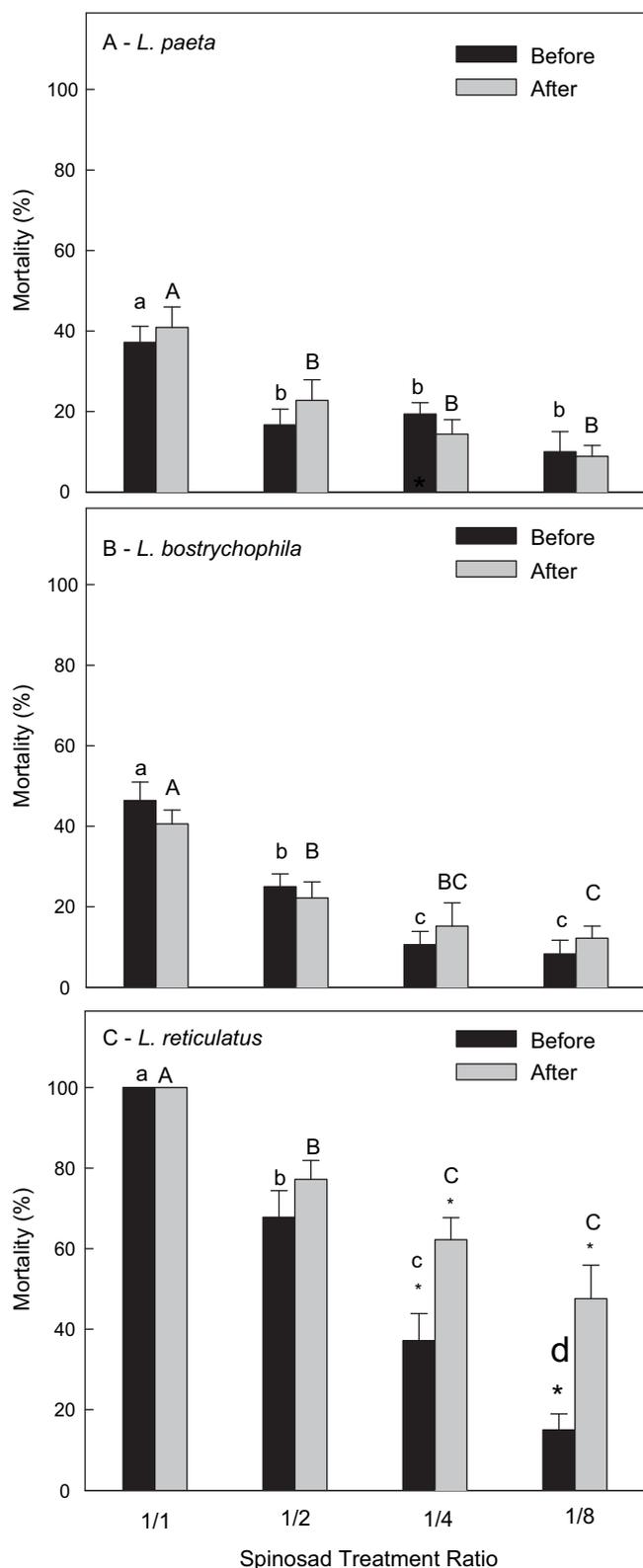
the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Arthur, 1992).

*Rhyzopertha dominica* was by far the most susceptible species in accordance with nearly all of the previous studies with spinosad (Fang et al., 2002; Nayak et al., 2005). In our study, all *R. dominica* adults were dead when the entire mass of wheat in the vials was treated, regardless of whether they were placed in the vials before or after the wheat. Also, *R. dominica* mortality was 83% or more in the surface layer treatments, provided that the adults had been released at the top of the grain mass. Consequently, when *R. dominica* adults penetrate a thin treated layer to colonize deeper, untreated areas of the wheat bulk, this species could be vulnerable to spinosad even during short exposure conditions. In the vials used in the tests, the width of the top one eighth of the grain mass can be considered as a two to three wheat kernel-deep layer, which means that beetles can easily reach the untreated wheat. While we do not know how long *R. dominica* were exposed in the treated layers, survival patterns indicate vertical movement occurred during the 14-day experimental period. However, when insects were already in the untreated layer, the results were completely different, suggesting little upward movement of *R. dominica* occurred during the 14-day exposure period. In another test utilizing vertical columns of wheat as the experimental units, Vardeman et al. (2007a) showed adult *R. dominica* were capable of penetrating a surface layer treated with diatomaceous earth (DE) and reproducing in the untreated wheat below the surface layer. Results from laboratory bioassays (Vardeman et al., 2007b) also indicated downward movement of *R. dominica* in untreated wheat and in wheat treated with DE. Surtees (1964) also reported downward movement of *R. dominica* released from the top of a grain mass. The lack of upward movement or mobility could indicate that a surface treatment of spinosad would not be effective if the wheat was already infested, even though *R. dominica* is very susceptible to spinosad.

Mortality of *S. oryzae* revealed a lower susceptibility to spinosad than *R. dominica*. Athanassiou et al. (2008) found that adults of *S. oryzae* were less susceptible than adults of *R. dominica* to spinosad dust, consistent with previous tests with liquid spinosad (Fang et al., 2002; Nayak et al., 2005). The results of our study showed a difference in mortality between weevils placed before or after the introduction of wheat in the vials. This could reflect greater downward movement from the surface of the wheat in the vials compared to upward movement from the bottom, which would account for the lower mortality of the weevils placed in the vials prior to the introduction of the treated wheat. Species of the genus *Sitophilus* are generally mobile and readily move throughout a grain mass (Surtees, 1964, 1965; Wakefield, 1995; Mvumi et al., 2006). However, there is some evidence of upward movement from deeper layers within the grain mass. Surtees (1964) showed upward movement of *Sitophilus granarius* (L.), while Mvumi et al. (2006) reported that *S. zeamais* was concentrated in the bottom layers of maize treated with DE. In our study, although the insects were confined to a small wheat column of only 3 cm in diameter, results show that, even then, the presence of untreated layers may prevent complete control of *S. oryzae*.



**Fig. 1.** Percentage mortality (mean ± SE) of *R. dominica* (A) and *S. oryzae* (B) adults in vials containing wheat totally treated with spinosad (1/1 spinosad) or wheat with the upper half, one fourth, or one eighth layer treated with spinosad. For each species within each spinosad treatment ratio, asterisks indicate significant differences in mortality if adults were introduced to vials before or after the introduction of the wheat, and different lower-case and upper case letters indicate a significant difference in mortality between spinosad treatment ratios in the before and after placements, respectively ( $P < 0.05$ , HSD test).



**Fig. 2.** Percentage mortality (mean  $\pm$  SE) of *L. paeta* (A), *L. bostrychophila* (B) and *L. reticulatus* (C) adult females on vials containing wheat totally treated with spinosad (1/1 spinosad) or wheat with the upper half, one fourth, or one eighth layer treated with spinosad. For each psocid species within each spinosad treatment ratio asterisks indicate significant differences in mortality if adults were introduced before or after the introduction of the wheat, and different lower-case and upper case letters indicate a significant difference in mortality between spinosad treatment ratios in the before and after placements, respectively ( $P < 0.05$ , HSD test).

There are few published reports regarding efficacy of spinosad against stored-product psocids. Nayak et al. (2005) found that an application rate of 1 ppm on wheat produced about 50% mortality of adults, although there was some reduction in progeny compared to untreated controls. Also, Nayak and Daghli (2006) reported poor control of *L. bostrychophila* and *Liposcelis decolor* (Pearman) with spinosad, while chlorpyrifos-methyl provided complete control either alone or in combination with spinosad. However, in the same tests, control of *L. paeta* and *Liposcelis entomophila* (Enderlein) was achieved only with combinations of spinosad with chlorpyrifos-methyl. According to the results of our test and those studies cited above, *L. paeta* and *L. bostrychophila* are very tolerant to spinosad, and this tolerance may not be related to individual strains, but may instead be a species-specific characteristic. In contrast, *L. reticulatus* was highly susceptible to spinosad. In our study, survival occurred when only the upper layer of the wheat was treated, and was reduced with increasing depth of the treated layer. Our results also suggest downward movement of the psocid species, as described in field studies by Throne et al. (2006).

If the initial presence of insects in stored wheat is low, it may take several months for an insect infestation to develop (Hagstrum, 1989). If only a single layer is treated at that stage (e.g. the top layer), it may be possible to reduce costs and total residues, and also provide protection for the bulk wheat mass. However, any delayed mortality that occurs when insects pass through this barrier may be an important factor, especially if oviposition occurs from parental adults that have passed through the treated layer. Our laboratory results indicate that even the short contact with a small spinosad-treated portion of the grain mass might be effective against *R. dominica*. However, results for *S. oryzae*, *L. paeta*, and *L. bostrychophila* show that these species may be able to pass through a treated layer and survive, depending on the depth of this layer. In these cases, it may be necessary to combine spinosad with an insecticide with a different mode of action that would increase immediate mortality.

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