Comparative Susceptibility of *Liposcelis bostrychophila* Badonnel and *Liposcelis decolor* (Pearman) (Psocoptera: Liposcelididae) to Spinosad on Wheat

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ABSTRACT In 2005 the United States Environmental Protection Agency registered spinosad as a grain protectant at 1 µg(AI)/g. The effectiveness of spinosad at 1 µg(AI)/g against many beetle and moth pests of stored grains is well established. In recent years, several psocid species have emerged as economically important pests in various stored-product environments in many countries, including the United States. Information on susceptibility of stored-product psocid species to spinosad is basically unknown, especially in the United States. In laboratory tests, we evaluated adult mortality and adult progeny production of two major stored-product psocid species, *Liposcelis bostrychophila* Badonnel and *Liposcelis decolor* (Pearman), exposed to untreated and spinosad-treated wheat. Adults of the two species were exposed to 12 spinosad concentrations ranging from 0 to 16 µg(AI)/g of wheat for 7 d to determine susceptibility and to estimate median lethal concentrations (LC_{50}). Adults of each species were exposed to 0, 0.25, 0.5, and 1 µg(AI)/g for 56 d to determine adult progeny production. In general, at all spinosad concentrations, *L. bostrychophila* was less susceptible than *L. decolor*. At 1 µg(AI)/g, *L. bostrychophila* adult mortality was only 33% whereas that of *L. decolor* was 88%. The LC_{50} value for *L. bostrychophila* was 4.49 µg(AI)/g and for *L. decolor* it was 0.22 µg(AI)/g. Spinosad at 1 µg(AI) /g completely suppressed adult progeny production of *L. decolor*; at this spinosad rate reduction in progeny production of *L. bostrychophila* was only 91% relative to production on untreated wheat. Our results suggest that spinosad applied to stored wheat at the labeled rate of 1 µg(AI)/g is effective against *L. decolor* but not *L. bostrychophila*.

KEY WORDS : Grain protectants, stored-product insects, psocids, tolerance

INTRODUCTION

Historically, psocids were sporadic pests and were considered only as secondary pests of stored-products (Nayak and Daglish, 2006). However, during the last decade, some species have emerged as economically important pests in various stored-product environments in many countries (Leong and Ho, 1994; Rajendran, 1994; Santoso *et al.*, 1996; Wang *et al.*, 2000; Nayak *et al.*, 2005; Nayak and Daglish, 2007; Opit and Throne, 2008). Psocid management in stored-product environments has been a challenge because several species of psocids often occur together in a storage facility and there are considerable variations in susceptibility to grain protectants among species. Some species are very tolerant to traditional chemical grain protectants

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while others are relatively susceptible (Nayak *et al.*, 1998, 2005; Nayak and Daglish, 2007). For this reason, several new classes of insecticides have been explored for managing psocid pests of stored grains (Nayak and Daglish, 2007).

One new insecticide product that has shown promise as an effective grain protectant is spinosad. Spinosad is an environmentally benign insecticide that is based on metabolites of a soil bacterium, *Saccharopolyspora spinosad* Mertz and Yao (Mertz and Yao, 1990). This insecticide has an unique mode of action with low mammalian toxicity compared with other chemical insecticides (Bret *et al.*, 1997; Salgado, 1998, Thompson *et al.*, 1997, 2000). It is effective against many beetle and moth pests associated with stored grain (Fang *et al.*, 2002; Huang *et al.*, 2007). In 2005 it was labeled at 1 μg(AI)/g as a grain protectant for managing stored-product insects in the United States (Subramanyam, 2006), and commercial formulations may be released in 2010. However, data to support use of spinosad for managing stored-product psocid pests is limited. Efficacy of spinosad against psocids has only been examined for four species in Australia, and notable variation in susceptibility has been reported (Nayak *et al.*, 2005; Nayak and Daglish, 2007).

During a field survey conducted during 2002 in Kansas, USA, high densities of psocid populations were observed in several wheat bins. These psocids were identified as *Liposcelis bostrychophila* Badonnel and *L. decolor* (Pearman). *Liposcelis bostrychophila* is a cosmopolitan species and one of the most economically important stored-product psocid pests in the world (Wang *et al.*, 2000; Nayak and Daglish, 2007). In contrast, *L. decolor* is a relatively new stored-product pest. However, this species has recently been reported as an emerging pest in several countries including Australia, China, Croatia, Spain, and the Czech Republic (Nayak *et al.*, 2002). The objectives of this study were to determine the susceptibility and progeny production of Kansas populations of the two psocid species exposed to spinosad-treated wheat. Information generated from this study can be used to determine if spinosad is also a good grain protectant for managing these two economically important stored-product psocids in the United States. In addition, the baseline data established from this study will be useful in monitoring changes in susceptibility of the two psocid species to this new grain protectant.

MATERIALS AND METHODS

Insect Sources and Culture

A large number of adults of *L. bostrychophila* and *L. decolor* were collected from round metal bins holding wheat near Abilene and Manhattan in northeast Kansas during 2002, respectively. Field-collected psocids were reared in 150-ml plastic containers containing ground brown rice. The containers were held in relative humidity (RH) controlled plastic boxes (50 × 33 × 33 cm) containing appropriate amount of glycerol solution for maintaining humidity levels of 75–85% throughout the experiments (Rees, 1994). The humidity controlled plastic boxes with insect cultures were then placed in growth chambers maintained at 28–30˚C with a 14:10 h (L:D) photoperiod. Laboratory bioassays with spinosad were started within three months after adult collections.

Source of Insecticide

Spinosad (SpinTor™ 2SC) containing 240 mg(AI)/ml was provided by Dow AgroSciences LLC (Indianapolis, Indiana, USA). Insecticide dilutions were made in distilled water for treating grain.

Source of Wheat

Hard red winter wheat was obtained from the Milling Laboratory in the Department of Grain Science and Industry, Kansas State University (Fang *et al.*, 2002). Wheat was frozen for 1 wk at -13ºC to kill any live insects. Wheat was cleaned, tempered and equilibrated to 13% moisture in an environmental growth chamber maintained at 28°C and 65% RH.

Insecticide Bioassays

Two independent tests were conducted in this study. The first test was a concentration-response bioassay for determining susceptibility and median lethal concentration providing 50% mortality (LC_{50})

of the two psocid species. Wheat kernels were treated with spinosad to provide nominal rates of 0 (control), 0.016, 0.031, 0.063, 0.125, 0.25, 0.5, 1, 2, 4, 8, and 16 µg(AI)/g. Another test was conducted to determine progeny production of the two psocid species on untreated wheat (0 µg(AI/g) and wheat treated with 0.25, 0.5 and 1 µg(AI)/g.

To achieve the appropriate rates for the bioassays, 500 g of wheat in each jar (0.95-liter capacity) were treated with 0.5 ml of the spinosad solution or distilled water. Jars were placed in a plastic drum (38-liter capacity), and this drum was tumbled on a ball-mill roller for 10 min to ensure uniform coverage of the insecticide solution on wheat kernels. Untreated wheat for both tests received aliquots of distilled water. After tumbling, 20 g of the treated wheat were placed in each 150-ml plastic container. Fifty mixed-age psocid adults were introduced into each plastic container. Infested containers were closed with wire-mesh lids and placed in the humidity controlled plastic boxes as described above. In the concentration-response bioassay, the number of dead adults was checked after 7 days. In the progeny production test, 20 g of untreated and spinosad-treated wheat were infested with 50 unsexed adults. The number of adult progeny produced was counted after 56 days. Many eggs and nymphs were found in untreated wheat. It was cumbersome to count these stages, so only adult progeny were recorded in this study. Each combination of psocid species and spinosad rate was replicated five times unless otherwise mentioned, and each replicate was treated separately.

Data Analysis

Adult mortality data, expressed as a percentage, on spinosad-treated wheat was corrected for control mortality (Abbott, 1925), and data were transformed to angular values before analysis to normalize heteroscedastic treatment variances (Zar *et al.*, 1984). The transformed mortality data were subjected to two-way analysis of variance (ANOVA) with psocid species and spinosad rate as the two main factors (SAS Institute, 2007). Treatment means were separated using LSMEANS tests at the $\alpha = 0.05$ level (SAS Institute, 2007). Corrected concentration-mortality data for both species were also subjected to probit analysis (Finney, 1971; SAS Institute, 2007) to estimate LC_{50s} and associated statistics.

Data on adult progeny production were transformed to log (x + 1) scale and subjected to two-way ANOVA using the GLM procedure to determine differences in progeny production between the two species and across the four spinosad rates at the $\alpha = 0.05$ level (SAS Institute, 2007). Mean progeny production differences between the two species and among the spinosad rates was determined using LSMEANS test. Although data were transformed for analyses, in the figure and tables untransformed means and standard errors are presented.

RESULTS

Adult Mortality of *L. bostrychophila* and *L. decolor* on Spinosad-Treated Wheat

The main effect of spinosad rate on adult mortality of the two psocid species was significant ($F = 80.01$; df = 10, 87; $P < 0.001$). Adult mortality of both insect species after 7 d increased as spinosad rate increased (Fig. 1). Differences in adult mortality were also significant between the two psocid species ($F = 308.35$; df = 1, 87; $P < 0.0001$). *Liposcelis bostrychophila* was considerably less susceptible to spinosad than *L. decolor*. Adult mortality of *L. bostrychophila* was significantly lower ($P < 0.005$) than that of *L. decolor* at all spinosad rates tested (Fig. 1). Mortality of *L. bostrychophila* was low (< 7%) at 0.06 to 0.25 µg(AI)/g and was only 33% at the recommended rate of 1 µg(AI)/g. At 16 µg(AI)/g, the highest rate tested, adult mortality of *L. bostrychophila* reached only 73%. In contrast, adult mortality of *L. decolor* was 64 and 88% at 0.25 and 1 µg(AI)/g, respectively, and reached 100% at rates ≥ 2 µg(AI)/g. The interaction of spinosad rate and psocid species was also significant ($F = 2.99$; df = 10, 87; $P = 0.0027$), because mortality of *L. bostrychophila* increased from 36 to 73% as the rates increased from 2 to 16 µg(AI)/g, while mortality of *L. decolor* at the four highest rates tested was 100%.

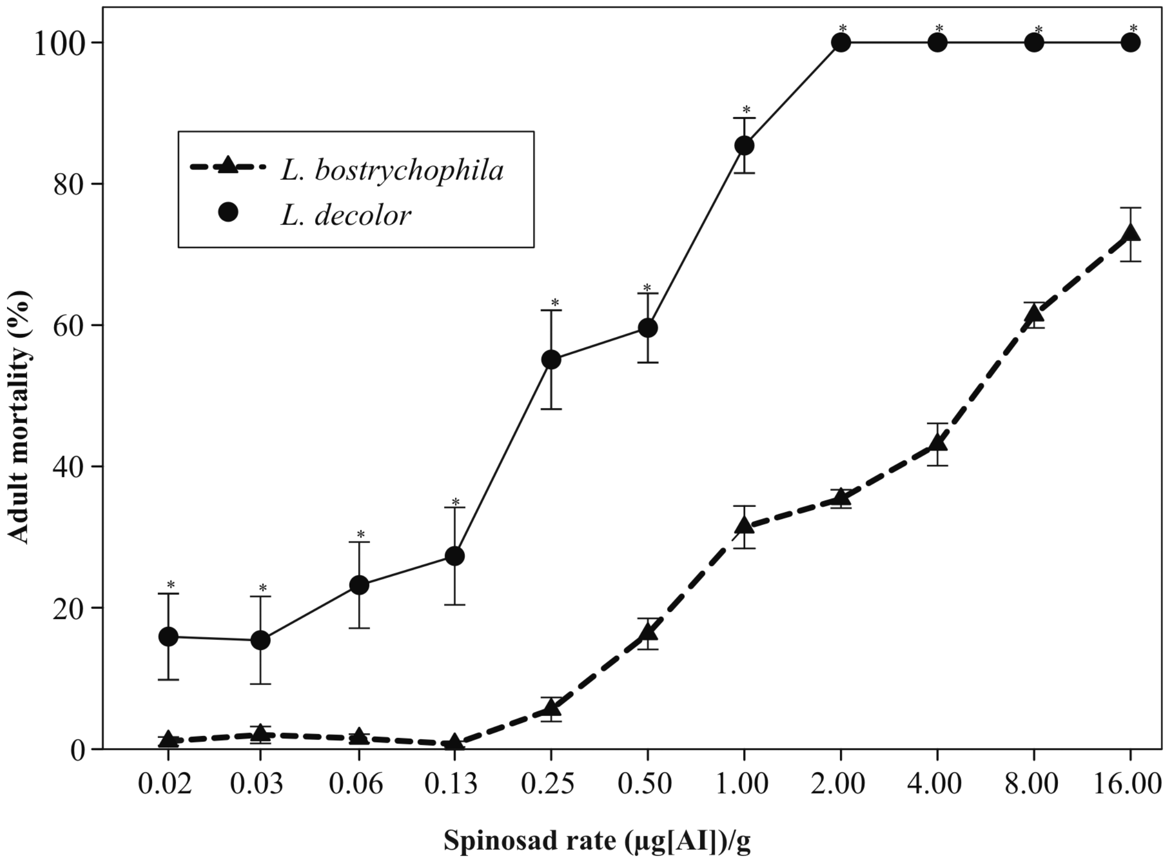

Fig. 1. Adult mortality (%mean ± SE) of *Liposcelis bostrychophila* and *Liposcelis decolor* after 7 d of exposure to wheat treated with various spinosad concentrations. For each spinosad concentration, mean values followed by an asterisk (*) are significantly different between the two species ($P < 0.05$; LSMEANS test).

Data used in the probit analysis included those recorded at the spinosad rates from 0.25 to 16 µg(AI)/g for *L. bostrychophila* and from 0.031 to 2 µg(AI)/g for *L. decolor*. The corrected adult mortality of *L. bostrychophila* ranged from 4.9 ± 2.1% (mean ± SE) at 0.25 µg/g to 72.8 ± 3.8% at 16 µg/g and the mortality levels of *L. decolor* ranged from 14.6 ± 6.7% at 0.031 µg(AI)/g to 100 ± 0.0% at 2 µg(AI)/g. The calculated LC_{50} value for *L. bostrychophila* was 4.49 µg(AI)/g with a 95%

Table 1. Probit regression estimates for *Liposcelis bostrychophila* and *Liposcelis decolor* exposed for 7 d to spinosad-treated wheat

Insect species	No. adults exposed[a]	Mean ± SE		LC_{50} (95% CI) (µg[AI]/g)[b]	χ^2 (df)	*P*-value[c]
		Intercept	Slope			
L. bostrychophila	1700	-0.71 ± 0.04	1.09 ± 0.06	4.49 (3.89 – 5.25)	36.78 (32)	0.2571
L. decolor	1750	1.02 ± 0.13	1.53 ± 0.15	0.22 (0.17 – 0.28)	180.3 (33)	< 0.0001

[a]Only 7 of the 11 spinosad concentrations were used to generate the probit regression estimates. The total number of insects used does not include psocids used in the control treatment.

[b]The extrapolated LC_{95} (95% CI) values for *L. bostrychophila* and *L. decolor* are 144.66 (95.95 – 239.46) and 2.58 (1.61 – 5.12) µg(AI)/g, respectively.

[c]The χ^2 values for goodness-of-fit of data were significant ($P < 0.05$) to the probit model.

confidence interval of 3.89–5.25 µg(AI)/g, which was 20-fold greater than that (0.22 µg(AI)/g) for *L. decolor* (Table 1). The difference was significant ($P < 0.05$) based on the lethal dose ratio test (Robertson and Preisler, 1992). The increased susceptibility of *L. decolor* is also reflected by the higher slope value (1.53) compared with *L. bostrychophila* (1.09).

Progeny Production of *L. bostrychophila* and *L. decolor* on Spinosad-Treated Wheat

On untreated control wheat, an average of 439 and 64 adult progeny of *L. bostrychophila* and *L. decolor* were produced, respectively (Table 2). The number of progeny produced of both species decreased with an increase in spinosad rate. In general, spinosad was less effective in suppressing progeny production of *L. bostrychophila* when compared with progeny suppression of *L. decolor*. For example, at the labeled rate of 1 µg(AI)/g 39 *L. bostrychophila* adults were produced, whereas no *L. decolor* adults were produced at this spinosad rate. The main effect of spinosad rate on progeny production was significant for the two psocid species ($F = 41.28$, df = 3, 32; $P < 0.0007$). Differences in progeny production were also significant between the two psocid species ($F = 303.21$; df = 1, 32; $P < 0.0001$). The interaction of spinosad rate and psocid species was also significant ($F = 9.86$; df = 3, 32; $P < 0.0001$), because progeny production decreased more significantly with an increase in spinosad rate for *L. decolor* than for *L. bostrychophila*.

DISCUSSION

The efficacy of spinosad for managing stored-product beetle and moth pests has been well established through laboratory and field tests (Fang *et al.*, 2002; Flinn *et al.*, 2004; Daglish and Nayak, 2006; Subramanyam, 2006; Huang *et al.*, 2007; Huang and Subramanyam, 2007; Subramanyam *et al.*, 2007; Daglish *et al.*, 2008). Limited published data exists on the effectiveness of spinosad for managing psocids associated with stored grain. Results of the current laboratory evaluation showed that the efficacy of spinosad against the two major stored-product psocid pests in the United States varied greatly. *Liposcelis bostrychophila* is much more tolerant to spinosad on wheat than *L. decolor*. The observed difference in susceptibility between the two species is likely due to physiological differenecs between the two species, because both psocid populations were collected from fields before spinosad was registered for use in stored-grains. In addition, commercial spinosad formulations will not be available for use on stored grains until 2010. Considerable variation in performance of spinosad against four stored-product psocid species was also reported from Australia (Nayak *et al.*, 2005; Nayak and Daglish, 2007). The baseline susceptibility data presented here could be used to monitor changes in susceptibility of these two psocid species to spinosad in Kansas.

Data from this study indicated that spinosad at 1 µg(AI)/g was marginally effective for managing *L. decolor* in stored wheat. Spinosad at 1 µg(AI)/g did not achieve 100% control efficacy (88% adult mortality) against *L. decolor* adults after 7 d, but completely prevented its progeny production. Compared to the published data from Australia, the Kansas population of *L. decolor* was relatively less tolerant to spinosad. Nayak *et al.* (2005) reported a

Table 2. Adult progeny production (mean ± SE) of *Liposcelis bostrychophila* and *Liposcelis decolor* on untreated and spinosad-treated wheat after 56 d

Spinosad rate (µg[AI]/g)	Mean ± SE of adult progeny/container[a]	
	L. bostrychophila[b]	*L. decolor*[b]
0	439.0 ± 48.3a	64.2 ± 12.5b
0.25	350.6 ± 20.9a	3.2 ± 2.2c
0.50	179.6 ± 26.5a	2.0 ± 2.0c
1	38.8 ± 12.1b	0c

[a]Means followed by different letters are significantly different ($P < 0.05$; by LSMEANS test).
[b]Each mean is based on $n = 4$ replications for *L. bostrychophila*; for *L. decolor* each mean is based on $n = 5$ replications.

mortality of 34.6% in an Australian population of *L. decolor* collected from the Darling Downs region of Queensland when exposed for 7 d to wheat treated with spinosad at 1 µg(AI)/g. After 14 d of exposure the mortality of *L. decolor* adults was 50%, and after 28 d it was 57.3%. Furthermore, at the 1 µg(AI)/g, the Australian population also produced significant number of progeny because of reduced adult mortality reported above.

Our results confirmed that spinosad at 1 µg(AI)/g is ineffective for managing *L. bostrychophila*, because adult mortality after 7 d was 33%. Progeny production of *L. bostrychophila* at 1 µg(AI)/g after 56 d was reduced by approximately 90% compared to progeny production on untreated wheat (Table 2). The 7 d mortality of *L. bostrychophila* adults observed in our study is similar to those observed in an Australian population of this species collected from the Darling Downs region of Queensland (Nayak *et al.*, 2005). In addition, two recent studies also showed that spinosad at 1 µg(AI)/g on wheat was also partially effective against *L. entomophila* and *L. paeta*, two other major stored-product psocid pests in Australia (Nayak *et al.*, 2005; Nayak and Daglish, 2007).

In summary, our study as well as the limited published information suggests that spinosad at the current recommended application rate (1 µg(AI)/g) may not provide a sufficient control for managing major psocid pests associated with stored grain. In addition, field studies have shown that more than 25% of the applied insecticides could be lost during application to the grain mass (Thomas *et al.*, 1987; le Patourel, 1992; Collins and Cook, 1998). Thus, wheat treated with the recommended application rate will likely be less than 1 µg(AI)/g immediately after application. Flinn *et al.* (2004) reported that the actual spinosad deposit levels on wheat immediately after treatment at the 1 µg(AI)/g application rate ranged only from 0.4–0.8 µg(AI)/g. Furthermore, uneven application and coating of insecticide on grain kernels could further reduce insecticide residues in some parts of the stored grains (Huang *et al.*, 2004). Therefore, field efficacy of spinosad on wheat at the labeled rate of 1 µg(AI)/g against psocid pests could be even much lower than that observed in the laboratory bioassays. Unfortunately, field data on efficacy of spinosad for managing stored-product psocid pests are not available.

The relatively low insecticidal activity of spinosad against stored-product psocid pests suggests that spinosad, if used, should be combined with other grain protectants to achieve sufficient control for managing psocid pests in stored environments. Nayak and Daglish (2007) reported that wheat treated with spinosad at 1 µg(AI)/g combined with chlorpyrifos-methyl at 5 or 10 µg(AI)/g could provide protection up to 3 months against four psocid species in Australia. Spinosad in combination with pirimiphos-methyl or chlorpyrifos-methyl has also been suggested for managing two major stored-product beetle pests, the red flour beetle, *Tribolium castaneum* (Herbst) and the confused flour beetle, *T. confusum* (Jacquelin du Val). These two *Tribolium* species are much less susceptible to spinosad compared to most other stored-product beetle and moth pests (Huang and Subramanyam, 2007; Subramanyam *et al.*, 2007). Results from the current study support the need to combine spinosad with other grain protectants for managing psocids associated with stored grain in the United States.

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