Evaluation of a Dry Spinosad Formulation on Two Extruded Pet Foods for Controlling Four Stored-Product Insects

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ABSTRACT The efficacy and persistence of spinosad, a commercial bacterial insecticide, against stored-grain insects on whole grains has been well documented through laboratory and field trials at 1 mg(AI)/kg, and at this rate it is currently labeled for use on grain. The effectiveness of spinosad applied to extruded pet products against stored grain insects is unknown. In laboratory tests, adults of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus); red flour beetle, *Tribolium castaneum* (Herbst); cigarette beetle, *Lasioderma serricorne* (Fabricius); and warehouse beetle, *Trogoderma variabile* (Ballion) were exposed to untreated chicken feed pellets and dry commercial dog food and those treated with 1, 3, 5, and 10 mg (AI)/kg of a dry spinosad formulation (0.5 per cent AI). Adults (25) were added to untreated or spinosad-treated chicken feed or dog food and examined after 7 and 14 days to determine mortality and after 49 days to determine adult progeny production. Chicken feed and dog food were unsuitable diets for *L. serricorne* and *T. variabile*, because of high mortality of adults in the control treatment. Progeny of *L. serricorne* were not produced on dog food because of 100 per cent adult mortality at all spinosad rates, but progeny were observed in chicken feed, and ≥ 5 mg(AI)/kg spinosad was necessary for effective progeny suppression. Very few progeny of *T. variabile* were observed on chicken feed and dog food because of high adult mortality of this species on the two pet foods. Mortality of *T. castaneum* and *O. surinamensis* adults on chicken feed and dog food at the highest rate of spinosad at 10 mg(AI)/kg was less than 80 per cent. On these two pet foods, suppression in progeny production of *T. castaneum* and *O. surinamensis* was evident at spinosad rates ≥ 3 mg(AI)/kg. Our results suggest that spinosad is not a viable product for use in these pet foods for management of the four insect species tested.

KEY WORDS: Dry spinosad, stored-product insects, pet food

INTRODUCTION

The pet food industry in the United States in 2004 was estimated at $14.7 billion and it is anticipated to reach $18 billion by 2008 (Barnes, 2005). Once packaged, these valuable commodities are moved throughout the marketing system from the processing plant to warehouses, trucks, retail stores, and consumer's homes (Hagstrum and Subramanyam, 2006). Dry pet foods are susceptible to stored-product insects at all points in this marketing system,

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particularly in warehouses and retail stores (Roesli *et al*., 2003). Packaging offers some protection from stored-product insect infestation. However, in many cases, insects are either able to chew through the packages or enter packages through natural openings and inadequate seals (Mullen and Mowry, 2006).

Insects that enter packages can be categorized as penetrators or invaders based on their mode of package infestation. Penetrators, such as the cigarette beetle, *Lasioderma serricorne* (Fabricius), and warehouse beetle, *Trogoderma variabile* (Ballion), bore through the layers of flexible packaging material. Invaders, such as the red flour beetle, *Tribolium castaneum* (Herbst) and sawtoothed grain beetle, *Oryzaephilus surinamensis* (Linnaeus) take advantage of existing openings (Mullen and Mowry, 2006).

The use of sanitation and residual insecticides in warehouses and retail establishments, where packaged pet foods are stored, may reduce the presence of stored-product insects, thereby reducing infestations within packages. However, as reported by Roesli *et al*. (2003) sanitation in these establishments is poor, and sometimes sanitation has little or no impact on stored-product insect populations (Nansen *et al.*, 2004). In such cases, the use of packaging materials or chemicals that retard odors emanating from pet foods (Sacharow and Brody, 1987; Mullen, 1994) and application of chemicals (repellents) to the package exterior (Hou *et al.*, 2004) may help in preventing infestation within packages. Another approach to manage stored-product insects may include coating the surface of pet food products with an insecticide that is innocuous to pets, or incorporating an insecticide into the pet food matrix. The effectiveness of the former approach was evaluated in this paper, by coating two extruded pet foods with a reduced-risk commercial bacterial insecticide, spinosad. Spinosad is environmentally benign, and has a low mammalian toxicity (Sparks *et al.*, 2001). It is effective against several stored-product insect species (Fang *et al*., 2002a,b; Toews and Subramanyam, 2003a,b), and is labeled at 1 mg(AI)/kg as a grain protectant (Anonymous, 2005). The spinosad used was a dry formulation and it was applied at different rates to dog food and pelleted chicken feed to control four economically-important stored-product insects.

MATERIALS AND METHODS

Pet Foods

The pelleted chicken feed was made at the Kansas State University pilot feed mill. The pellets were composed primarily of corn (68 per cent by weight); soybean meal (25 per cent); and salt, soy oil, limestone, and vitamins/minerals (7 per cent). The Purina® Dog Chow®, a commercial dry dog food, was made up of 36 ingredients: ground yellow corn, poultry by-product meal, animal fat preserved with mixed-tocopherols (a form of Vitamin E), corn gluten meal, brewer’s rice, soybean meal, animal digest, calcium carbonate, calcium phosphate, salt, potassium chloride, L-Lysine monohydrochloride, choline chloride, zinc sulfate, Vitamin E supplement, zinc proteinate, ferrous sulfate, added color (Red 40, Yellow 5, Blue 2, Yellow 6), manganese sulfate, manganese proteinate, niacin, Vitamin A supplement, copper sulfate, calcium pantothenate, copper proteinate, garlic oil, pyridoxine hydrochloride, Vitamin B-12 supplement, thiamine mononitrate, Vitamin D-3 supplement, riboflavin supplement, calcium iodate, menadione sodium bisulfite complex (source of Vitamin K), folic acid, biotin, and sodium selenite (Nestle Products, 2006).

Treatment of Pet Foods with Spinosad

Chicken feed and dog food were initially kept in a freezer (-13°C) for one week to kill any living insects that might be present in these formulations. Approximately 100 g of chicken feed or dog food, free of fines, were placed in 0.45-litre glass jars. Lids for jars were fitted with 7-cm filter paper and wire mesh screens (250 ìm openings) for ventilation. Jars with chicken feed or dog food were placed in a growth chamber at 28°C and 65 per cent RH for one week to equilibrate the product moisture. This equilibration resulted in 8.7 per cent moisture content for the chicken feed and 7.6 per cent moisture content for the dog food, as determined by a modified version of an official method (AACC 2000; C. Reed, unpublished data). A dry formulation of spinosad (0.5 per cent AI; Dow AgroSciences, Indianapolis, Indiana), that is not yet commercially available, was applied to each 100-g lots in jars and admixed for 1 min by manual shaking to obtain nominal rates of 1, 3, 5, and 10 mg(AI)/kg. Untreated chicken feed and dog food in jars served as the control treatment.

Insects and Pet Food Infestation

The four insect species used in tests included *L. serricorne*, *T. castaneum*, *O. surinamensis*, and *T. variabile*. Twenty five unsexed adults of mixed ages of a species were separated from cultures maintained in the Department of Grain Science and Industry, Kansas State University, and introduced into each jar. Separate jars were used for different species. After infestation, jars with untreated and spinosad-treated chicken feed and dog food were closed with lids and held at 28°C and 65 per cent RH for assessing adult mortality and progeny production. A separate set of jars were used for mortality and progeny production assessments.

Mortality Assessment

After 7 d, jars were examined to count live and dead insects. Insects were considered dead if they did not respond to gentle prodding with a camel-hair brush. After counting, all insects and pet food samples were discarded. The number of dead insects out of the total exposed was expressed as a percentage and these percentages, for data analysis, were transformed to angular values (Zar, 1984).

Progeny Production

Larvae, pupae, and adults produced from the initial 25 adults originally introduced into each jar were counted after 49 days. From this number, the original 25 adults were subtracted to obtain the actual number of progeny produced. Progeny counts (x) were transformed to $\log_{10}(x + 1)$ scale for analysis.

Experimental Design and Statistical Analysis

A completely random design was used for all tests. Each pet food-species-rate combination was replicated three times for mortality assessment or progeny production, except for *T. variabile* in dog food tests where six replications were used. Data were analyzed separately by pet food and species using a one-way analysis of variance (ANOVA) to determine differences among spinosad rates (SAS Institute, 1999), because differences in susceptibility among stored-product insects on various substrates has been well documented (Fang *et al*., 2002a; Toews and Subramanyam, 2003b; Getchell, 2006). Treatment means were separated using Fisher's protected least significant difference test at $\alpha = 0.05$ (SAS Institute, 1999).

RESULTS

Mortality Responses of Adults on Chicken Feed and Dog Food

Mortality responses of adults of the four insect species on chicken feed are shown in Fig. 1. Mortality of *L. serricorne* adults was 69 per cent on untreated feed and differences among the spinosad rates (including control) were not significant (F = 2.11; df = 4, 10; $P > 0.154$). However, for *T. castaneum*, *O. surinamensis*, and *T. variabile* mortality tended to increase with an increase in spinosad rate (F, range among species = 4.34-16.03; df = 4, 10; $P < 0.0272$).

Irrespective of the exposure period, all *L. serricorne* adults died on dog food (Fig. 2). The mortality of *O. surinamensis* adults was higher on dog food treated with 1 to 10 mg(AI)/kg compared with controls, but differences among treatments were not significant (F = 3.46; df = 4, 9; $P > 0.056$). The mortality of *T. variabile* adults in spinosad treatments (99 per cent) was significantly different from control (83 per cent) (F = 12.92; df = 4, 25; $P < 0.0001$). Mortality of *T. castaneum* adults increased with an increase in spinosad rate (F = 19.50; df = 4, 10; P = 0.0001), and mortality at the highest rate was only 69 per cent.

Progeny Production on Chicken Feed and Dog Food

The progeny production of each of the four species varied among the treatments (F, range among species = 3.78-21.06; df = 4, 10; $P < 0.04$). Progeny production decreased with an increase in spinosad rate for both *L. serricorne* and *T. castaneum*, and more than 96 per cent reduction was observed at 5 and 10 mg(AI)/kg (Fig. 3). This trend was not

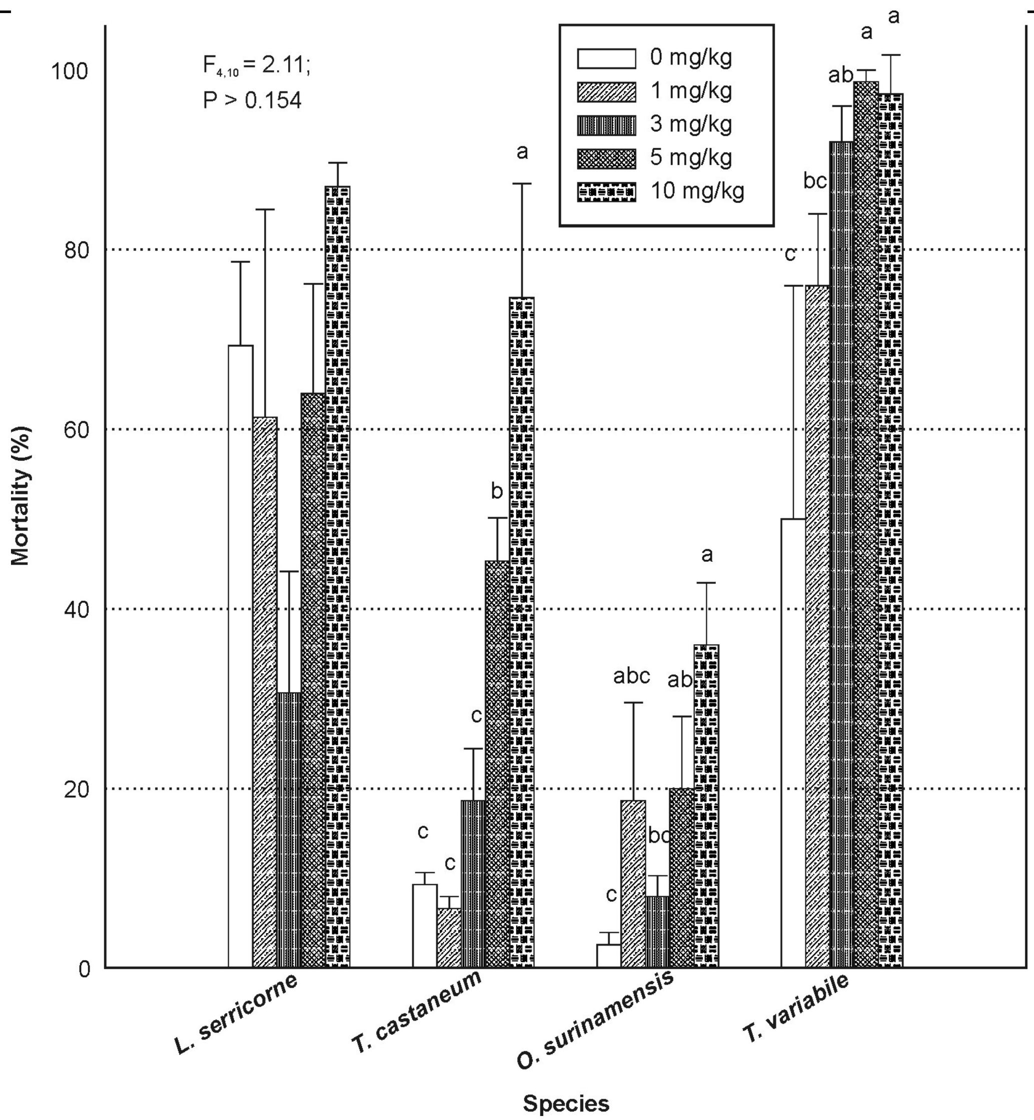

Figure 1. Mortality (% mean + SE) of adults of four insect species exposed for 7 days to untreated and spinosad-treated pelleted chicken feed. Means within a species followed by different letters are significantly different (*P* < 0.05; by Fisher's protected least significant difference test).

observed with *O. surinamensis* and *T. variabile,* and interestingly, for both these species more progeny were produced on chicken feed treated with 1 mg(AI)/kg spinosad than any other treatment but differences between the control and a majority of the spinosad treatments were not significant.

Progeny of *L. serricorne* were not observed on dog food (Fig. 4), probably because of 100 per cent mortality of adults within 7 days (see Fig. 2). Dog food was also a poor substrate for *T. variabile* (F = 0.85; df = 4, 25; $P > 0.0576$), because very few progeny were produced in the control (0.8 insects) and spinosad treatments (0 to 2.5 insects). More progeny of *T. castaneum* were produced on untreated dog food (447 insects) when compared with production of *O. surinamensis* (25 insects), but for both species, there was a significant decrease in progeny production with increasing spinosad rate (F = 34.57; df = 4, 10; $P < 0.0001$ for *T. castaneum* and F = 7.81; df = 4, 10; $P = 0.004$ for *O. surinamensis*).

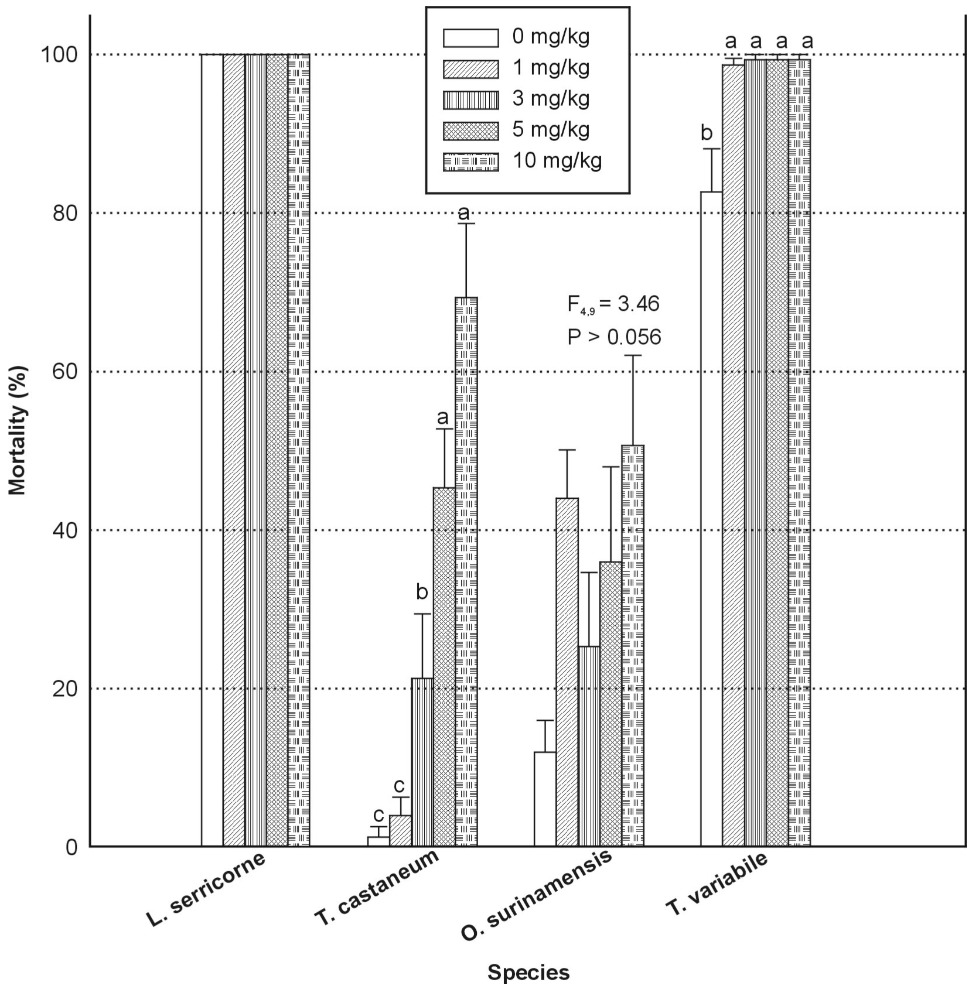

Figure 2. Mortality (% mean + SE) of adults of four insect species exposed for 7 days to untreated and spinosad-treated dog food. Means within a species followed by different letters are significantly different (*P* < 0.05; by Fisher's protected least significant difference test).

DISCUSSION

Blanc *et al*. (2004) reported on the efficacy of spinosad incorporated into an artificial diet against *L. serricorne* larvae at 50 mg(AI)/kg. The rate used and method of insect exposure by Blanc *et al*. (2004) were different from our study. Adults of *L. serricorne* on chicken feed were not susceptible to spinosad, because the mortality of adults in the control and spinosad treatments was essentially similar. Spinosad rates greater than 10 mg(AI)/kg may be necessary to obtain effective insect kill of this species on chicken feed. However, the decreased progeny production of *L. serricorne* on spinosad-treated chicken feed compared with untreated feed suggests that spinosad is effective against larvae. In contrast to chicken feed, dog food is an unsuitable substrate for *L. serricorne* because of complete adult mortality at all rates and the lack of progeny production. A similar, but less adverse effect was observed with *T. variabile*. It is plausible that one or several of the ingredients in the commercial dog food formulation may have contributed to this adverse effect. The mortality of *T. castaneum* and *O. surinamensis* adults was less than satisfactory on both the chicken feed and dog food, even at 10 mg(AI)/kg. These species are generally less susceptible to spinosad than other stored-product insects on various stored grains at the labeled rate of 1 mg(AI)/kg (Fang *et al*., 2002a,b;

Getchell, 2006). Decreased progeny production of *T. castaneum* on chicken feed and dog food, especially at 5 and 10 mg(AI)/kg suggests increased susceptibility of larvae to spinosad. This observation is consistent with previous findings on stored grain (Fang *et al*., 2002a; Flinn *et al*., 2004; Nayak *et al*., 2005). Decreased progeny production of *O. surinamensis* on stored grains at 1 mg(AI)/kg also suggests increased susceptibility of larvae (Fang *et al*., 2002a; Toews and Subramanyam, 2003a; Nayak *et al*., 2005). In our tests this effect was observed with dog food, especially at 3 to 10 mg(AI)/kg, and with chicken feed at 10 mg(AI)/kg.

The application of dry spinosad to chicken feed and dog food at the various spinosad rates did not alter the appearance of these foods. The adherence of dry spinosad particles to the chicken feed and dog food was not studied in this investigation, but the differences observed in species responses on these two pet foods could be related to adhesion of dry spinosad particles and their availability to insects. The effects observed here on the species,

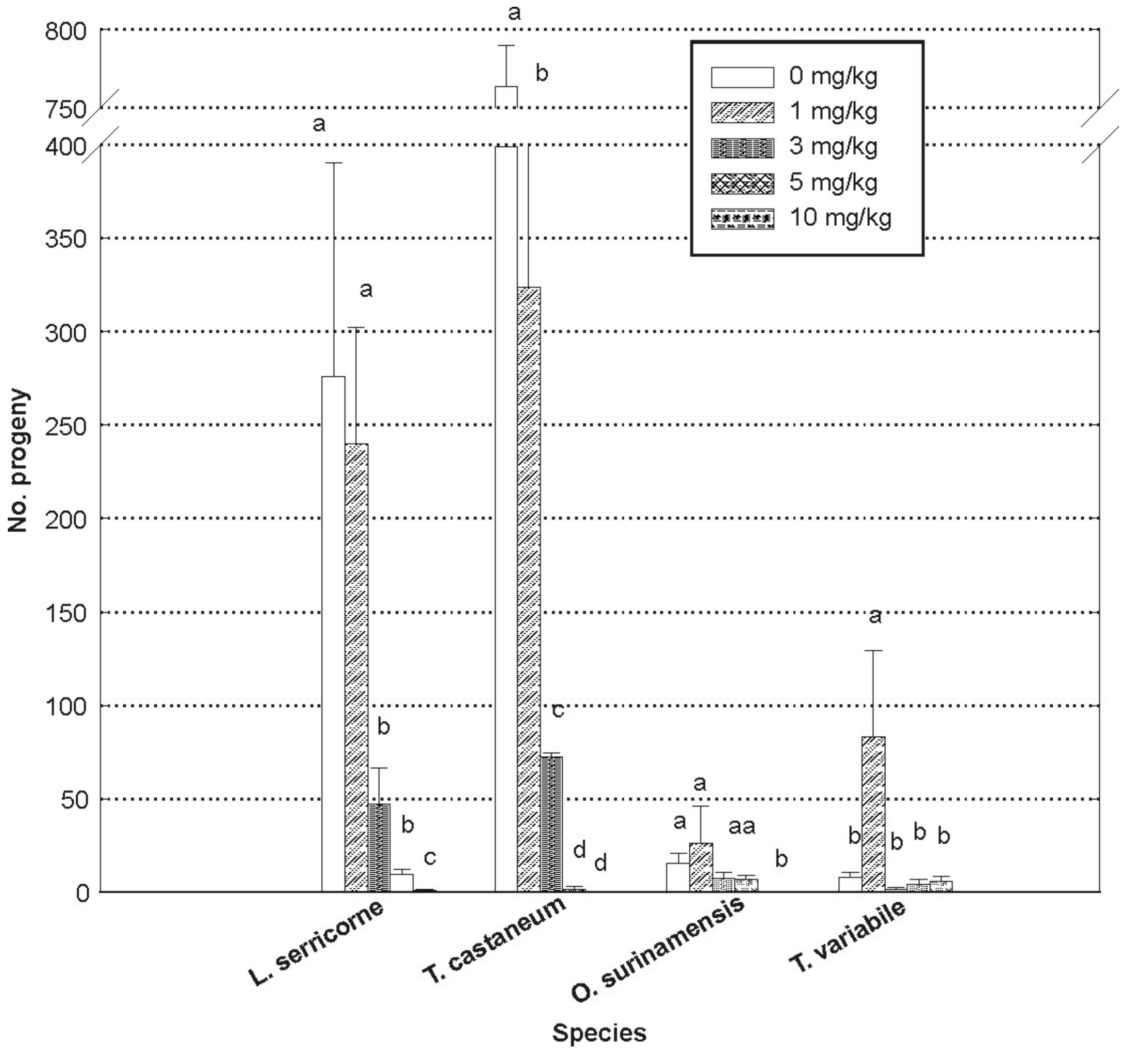

Figure 3. Number (mean + SE) of progeny produced by four insect species after 49 days on untreated and spinosad-treated pelleted chicken feed. Means within a species followed by different letters are significantly different (P < 0.05; by Fisher's protected least significant difference test).

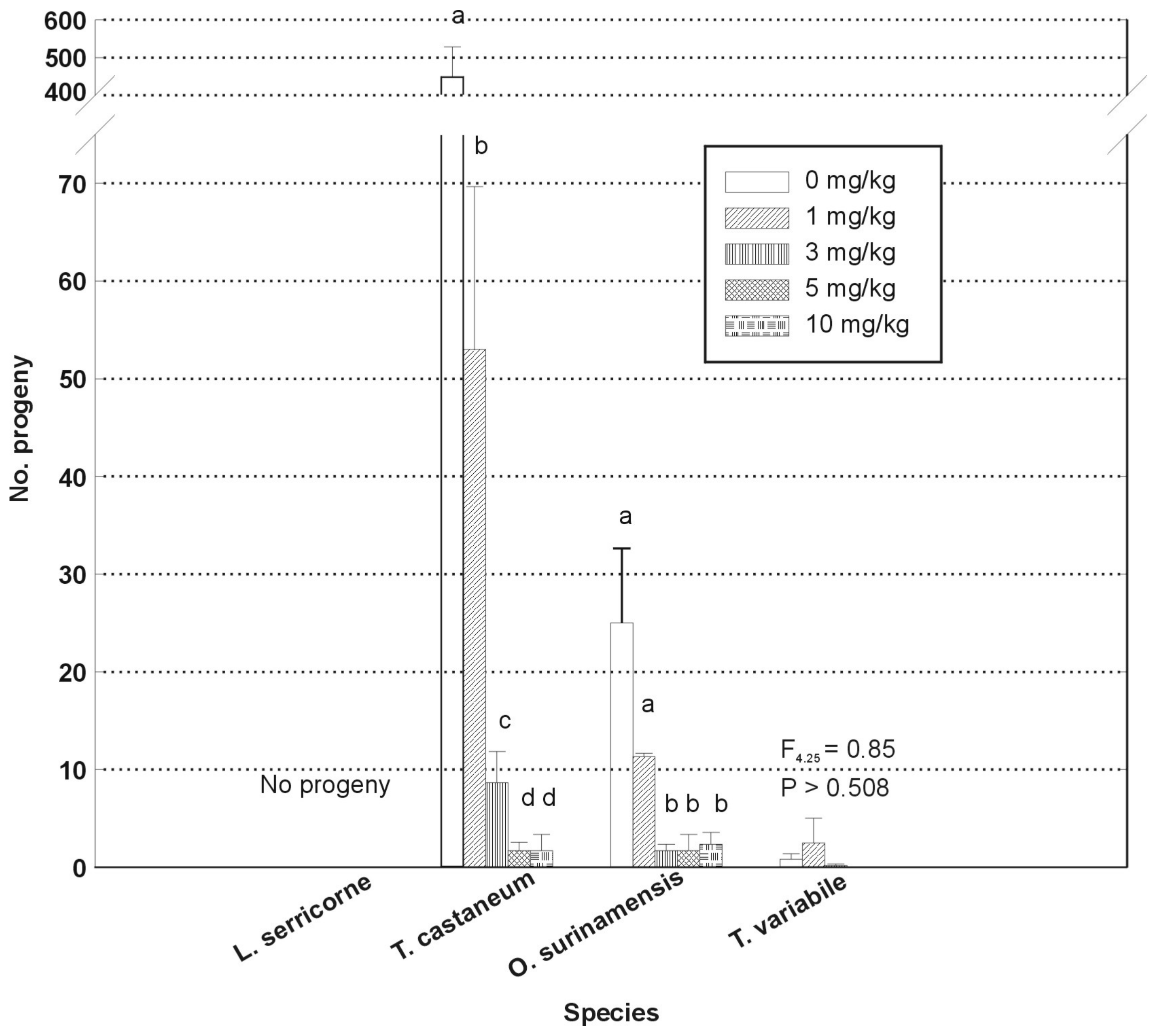

Figure 4. Number (mean + SE) of progeny produced by four insect species after 49 days on untreated and spinosad-treated dog food. Means within a species followed by different letters are significantly different (*P* < 0.05; by Fisher's protected least significant difference test).

especially *T. castaneum* and *O. surinamensis*, was consistent with that seen on stored grains. The dry spinosad formulation is effective at the labeled rate of 1 mg(AI)/kg on stored grains against several stored-product insects (Mutambuki *et al*., 2002; Getchell, 2006). However, surface application of dry spinosad to chicken feed and dog food for managing stored-product insects is not a viable option, because of the high rates required (3 to 10 mg(AI)/kg). Incorporation of dry spinosad throughout the matrix of the chicken feed and dog food may result in better distribution and availability to insects as they consume the spinosad/pet food encapsulate. Production of spinosad/pet food encapsulate is currently being explored using extrusion technology.

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