EFFICACY OF SPINOSAD FORMULATIONS TO CONTROL STORED GRAIN INSECTS

By

JAMES BARRETT ROBERTSON

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Thesis Approved:

Jack Dillwith, Thesis Adviser

Thomas W. Phillips, Co-advisor

Jim T. Criswell, Committee Member

Gordon Emslie, Dean of the Graduate College

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NOMENCLATURE

AI	Active ingredient
l	Liter(s)
ml	Milliliter(s)
g	Gram(s)
m	Meter(s)
cm	Centimeter(s)
°C	Degrees Celsius
RH	Relative humidity
L:D	Light : Day
h	Hours
mg/kg	Milligrams per kilograms
ppm	Part per million
c-m	Chlorpyrifos-methyl
>	Greater than
<	Less than
≈	Approximately
%	Percent

CHAPTER I

BACKGROUND INFORMATION AND RESEARCH OBJECTIVES

Background Information

The use of insecticides in attempts to inhibit unwanted invasions of insects is a practice that has been carried out for over a thousand years, although insecticide applications were not very effective against insects before the mid 1800s. The modern pesticide era was initiated when insecticidal properties of the chemical dichlorodiphenyltrichloroethane (DDT) were discovered in 1939. Organophosphorus insecticides were first developed in the 1940s, and many other chemicals were introduced to combat insect pests in decades following. Awareness of the destructive potential of pesticides towards non-target organisms was influenced after Rachel Carson's book *Silent Spring* was published in 1962. Following the establishment of the Environmental Protection Agency in 1970 the use of many pesticides was banned, and regulations for registering pesticides have since become stricter (Ware and Whitacre 2004).

Insect resistance to an insecticide occurs when exposure to a toxic substance results in a genetically-based reduced sensitivity to that substance (Ware and Whitacre 2004), and resistance has been a major concern in control of stored product insects. Resistance spreads when individuals that survive exposure to a toxic substance pass on resistance genes to succeeding generations of progeny (Subramanyam and Hagstrum 1995). The ultimate result of passing resistance genes is an increased number of resistant individuals within an infesting population. Resistance to chlorpyrifos-methyl in stored grain insects has been documented in *Rhyzopertha dominica* (Zettler and Cuperus 1990,

Subramanyam and Hagstrum 1995), and *Plodia interpunctella* (Subramanyam and Hagstrum 1995). *Tribolium castaneum* and *T. confusum* have been reported as resistant to a variety of insecticides, including chlorpyrifos-methyl (Zettler 1991).

Tolerance, in context to resistance, describes an ability that is naturally possessed by an individual to survive normally effective doses of an insecticide (Ware and Whitacre 2004). Tolerance to chlorpyrifos-methyl in stored grain insects has been demonstrated in *Oryzaephilus surinamensis* (Subramanyam et al. 1989, Subramanyam and Hagstrum 1995), in which the species was described as being "naturally tolerant."

Cross-resistance occurs when individuals of a population that have adaptively evolved resistance to one insecticide also show resistance to another insecticide with a similar mode of action. However, this concept of cross-resistance should not suggest that resistance in an insect population toward a particular insecticide automatically accrues resistance in the same population toward another insecticide of the same chemical class or with the same mode of action.

One practice employed to prevent insect resistance to insecticides in stored grain is to apply combinations of insecticides as mixtures. Though other practices involving multiple pesticide applications do exist, applications of insecticides in combination is theoretically a more effective means of managing resistance (Subramanyam and Hagstrum 1995). Using two insecticides with different modes of action as a mixture application in stored grain may increase the spectrum of insecticidal activity toward a broader range of insect pests, meanwhile avoiding the possible development of cross-resistant pest populations. Consequently, the application of pesticide mixtures raises the possibility for antagonism between insecticides (Daglish 1998, Desmarchelier 1977). A

dual insecticide antagonism was witnessed in a study with two insecticides, chlorpyrifos-methyl and Spinosad, applied to wheat, and demonstrated in beetle species *O. surinamensis*, *Cryptolestes ferrugineus*, and *Sitophilus oryzae* (Phillips and Bonjour, *personal communication*).

The insecticide Spinosad owes its name and insecticidal activity to the two naturally occurring active ingredients, spinosyn A and spinosyn D, which differ only slightly in structure at a molecular level (Bartholomaes 2001). The basic structure of spinosyn molecules is two sugars, a rhamnose sugar and a forosamine sugar, located on opposite ends of a macrolide ring (Saunders 1997). Spinosyns are a class of fermentation products synthesized by the bacterial actinomycete species *Saccharopolyspora spinosa* (Bartholomaes 2001). The genus name *Saccharopolyspora* is a Latin conglomerate that means "sugar loving many spores" (Thompson 1997). These bacteria were named according to the bacterial cultures in bagasse that remain following sugar cane extraction; characteristics of the genus were first described with the representative bacterium, *S. hirsuta* (Lacey 1975). *S. spinosa* was aptly named in reference to the bacteria's spiny outer sheath (Mertz and Yao 1990). A single strain of *S. spinosa* was collected in the Virgin Islands in a soil sample from an old sugar mill rum still site in 1980, and recognized as a new species of bacterium a decade later (Mertz and Yao1990). Early insect assays involving the effects of the fermentation products produced by *S. spinosa* indicated insecticidal properties with selectivity toward test insects in the orders Lepidoptera and Diptera (Thompson et al. 1997). Spinosad was introduced to the global market in 1997 by DowElanco as the first insecticide in a new class termed Naturalyte products (Thompson et al. 1997, Bret et al. 1997).

Spinosad is particularly important because of its novel mode of action, reduced risk to natural environments, and its low risk to human health. Additionally, Spinosad is characterized as a relatively broad-spectrum insecticide, but also exerts limited effects on non-target species. Spinosad expresses its insecticidal activity most notably as a nicotinic acetylcholine receptor agonist, though it does not work in the same fashion as nicotine neonicotinoids (Crouse et al. 1999). Spinosad has also been observed to exhibit effects on the GABA-gated chloride channel to an extent that is not well understood (Crouse et al. 1999), and has also been reported as having some ovicidal properties (Bret et al. 1997). Having a novel mode of action is advantageous for Spinosad use because there should be little risk of cross-resistance developing between Spinosad and other insecticides. Spinosad gains entry into an insect pest through two possible routes, either through the cuticle upon contact, or through the mouth upon ingestion. Two effective modes of entry is a characteristic that many other biologically derived insecticides do not possess, and may partially explain why Spinosad has been describe as having a rapid killing rate (Bret et al. 1997).

The low environmental risks posed by Spinosad were assessed by Saunders and Bret (1997). Tests showed that ultraviolet radiation from sunlight can break down the active ingredients in Spinosad within 16 days. The rapid photodegradation was observed to occur in water and on soil and plant surfaces. Beneath soil surfaces and away from sunlight, Spinosad was found to undergo biotic degradation from soil microbes. Movement of Spinosad throughout the environment is limited because it is non-volatile, and soil leaching is so slow that ground water contamination risks are of little concern. Research also showed that complete breakdown of the active spinosyns yielded the two

sugars, rhamnose and forosamine, which commonly occur throughout nature, and the macrolide ring was found to further degrade to form carbon dioxide and water (Saunders and Bret 1997).

Concerns about Spinosad in the environment, though relatively few, do exist pertaining to toxicity of certain non-target species. Spinosad was determined to be moderately toxic in aquatic environments to certain fish and other organisms (Bret et al. 1997), but if used properly risks of aquatic contamination can be avoided. High rates of activity against beneficial Hymenoptera have been observed in laboratory tests with honey bees, although this was less evident in field trials (Bret 1997), and with parasitoid wasps (Bret et al. 1997), some of which are natural enemies of certain stored grain pests (Toews and Subramanyam 2004). The use of Spinosad in grain storage bins would be of little concern to the aquatic life and honey bees as long as treated grain is safely stored in some form of enclosure. Parasitoids of grain pests would only be affected if pest infestations were extreme enough to warrant treatment with insecticides.

Spinosad has become widely used, and by 2002 it was registered as an insecticide for as many as 250 different crops (Subramanyam et al. 2002). In 2005 the Environmental Protection Agency (EPA) approved grain treatment levels of 1 mg active ingredient per kg grain (U.S. EPA 2005). The manufacturer of Spinosad, Dow AgroSciences, has yet to release a stored grain product for sale at this date because they are awaiting approval of allowable grain residue levels in one or more countries to which he U.S. exports grain. Much research has been conducted using Spinosad as a stored grain protectant, and many positive results have been observed. Studies involving *R. dominica*, a notorious pest in stored grain, have concluded that near complete control can

be achieved at an application rate of 1 mg/kg (Fang et al. 2002a, b, Nayak et al. 2005, Daglish and Nyak 2006). Other stored grain pest species found to be highly susceptible to Spinosad at this rate of application include larva of *P. interpunctella* (Fang 2002a) and *T. castaneum*, *Cryptolestes ferrugineus* (Huang et al. 2004), and *Sitophilus oryzae* after 42 days of exposure (Nayak et al. 2005).

Research Objectives

The objectives for the research conducted for this thesis were to answer the following questions.

1. Is there a difference in efficacies between liquid and dry formulations of Spinosad against stored grain insects when applied to stored wheat?
2. Can the previously inferred antagonism between Spinosad and chlorpyrifos-methyl be demonstrated with stored grain insect pest species not previously studied?
3. Is the antagonistic effect between Spinosad and chlorpyrifos-methyl due to some sort of chemical degradation of one or more of the active spinosyn ingredients as a result of methods of mixing the two insecticides?

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CHAPTER II

EFFICACIES OF THREE DIFFERENT FORMULATIONS OF SPINOSAD FOR CONTROLLING INSECT PESTS OF STORED WHEAT

Introduction

Following the discovery of *Saccharopolyspora spinosa* in 1990 (Mertz and Yao 1990) and the insecticidal properties of its metabolites (Thompson et al. 1997) spinosyn A and D, a new class of naturally-derived insecticides became available for use on crops worldwide in 1997 (Bret et al. 1997, Thompson et al. 1997). Experimental use of Spinosad on stored grain was granted by the Environmental Protection Agency (EPA) in 2002 (Huang et al. 2004), and in 2005 the EPA registered Spinosad for use as a protectant of stored grain, however a commercial product has not yet been marketed. The registered rate of application for Spinosad products on stored grain is 1 mg of active ingredient (AI) per 1 kg of commodity, or 1 part per million (ppm). Since ratios of spinosyns A to D are about 6 or 7:1 (Bartholomaes 2001) regardless of formulation, it makes sense to assume that Spinosad products applied at this rate should provide similar levels of protection against stored grain pests.

Several studies have focused on the efficacy of Spinosad for use on stored commodities to control insect pests since the late 1990s, but very few have been conducted to compare efficacies of different formulations of Spinosad on stored grain insects. Studies by Getchell (2006), in which efficacies of different formulations were compared, showed no difference between particular dry and liquid formulations on stored commodities such as wheat, sorghum, and corn against two species of stored grain insects. These studies only compared two different Spinosad formulations. Further tests comparing efficacies of other formulations that may eventually be marketed for use as

stored grain protectants are necessary to evaluate their efficacy and market potential. The purpose of the following experiment was to compare residual insecticidal activity of three different formulations of Spinosad against four species of stored grain pests on treated wheat stored under quasi-field conditions.

Materials and Methods

Treatments. Previously untreated hard red winter wheat was treated with three different formulations of Spinosad, a combined organophosphate and pyrethroid formulation as a positive control, and a water only negative control. Commercial formulations were provided directly by the manufacturer. The three Spinosad formulations included a liquid formulation containing 8.66% AI in the concentrate (Secure Liquid, Gustafson LLC, Plano, TX), referred to here as Spinosad Liquid, and two dry formulations; one containing 0.5% AI (Secure Dry, Gustafson LLC, Plano, TX), called Spinosad dry, and a dust formulation containing 0.125% AI, called Spinosad dust (Dow AgroSciences, Indianapolis, IN). The Spinosad dust formulation was a fine white powder, while the dry formulation was more course in consistency. The AI content for the positive control concentrate was 21.60% chlorpyrifos-methyl and 3.70% deltamethrin, which was the commercial product called STORCIDE II® (STORCIDE II, Gustafson LLC, Plano, TX). Each treatment was applied to four replications and each replication consisted of a 1-gallon, approximately 3,700 ml, glass jar containing 2000 g of wheat. Gallon jars were emptied of wheat prior to each individual treatment, and treatments were applied to the inner surface of each respective jar. For the two dry Spinosad formulations, 1.5 ml of water was first applied to the inner surface of each jar, and then the powdered treatment

was applied evenly over the moist surface so it would stick to the jar's interior. All treatments involving Spinosad formulations were applied at a rate of 1 mg/kg, and the STORCIDE II was applied at a rate of 3 mg/kg chlorpyrifos-methyl and 0.5 mg/kg deltamethrin. Applications of water only controls were also made using 1.5 ml of water applied to the inner surface of an empty 1 gallon jar. Following each application, 2000 g of wheat was placed into the 1 gal jars, and then each jar was rotated on its sides for twenty full rotations, and then set aside for approximately 20-30 minutes. Jars were again rotated in the same manner until no liquid or dry materials clung to the inner surface, after which jars were rotated and additional 40 times to thoroughly mix the treatments with the grain kernels as much as possible.

Storage. Treated 1 gal jars of wheat were stored in boxes that were placed in a metal storage container located outdoors with no artificial climate control. The metal storage container had dimensions of approximately 1.8 m tall, 1.8 m wide and m in length, was used to mimic the temperature changes and other environmental conditions that would typically occur in a metal grain bin.

Bioassays. Bioassays of treated wheat were conducted at 0 weeks post treatment (within 24 h of treatment), 16 weeks post treatment, 32 weeks post treatment, and 48 weeks post treatment using Coleoptera pest species *Rhyzopertha dominica* (Bostrichidae), *Sitophilus oryzae* (Curculionidae), and *Tribolium castaneum* (Tenebrionidae) and the Lepidoptera pest species *Plodia interpunctella* (Pyralidae). All insect species tested were reared in colonies at Oklahoma State University. When preparing bioassays for the beetle species, 100 g of treated wheat was placed into a 237 ml glass canning jar using all whole wheat grains for *R. dominica* and *S. oryzae*, and 95 g of whole grains plus 5 g of crushed grains

for *T. castaneum*. Fifty un-sexed adults of each species were placed into each of the small jars, lids for jars were made of a piece of mesh screen sandwiched between two pieces of 7.0 cm filter paper fitted into a canning jar lid ring, and jars were incubated in a growth chamber at a constant temperature of 28ºC, approximately 40-60% RH and a photoperiod of 16:8 h (L:D) for 7 days. Adults were removed from the bioassay jars on day 7 by sifting and adult mortality was assessed. Following adult sifting, all wheat and small debris were placed back into the bioassy jars, and were returned to the growth chamber for an additional 49 days of incubation to allow a new generation of adult progeny to develop.

Bioassays for *P. interpunctella* larvae were set up in 20 ml glass vials containing 2.5 g whole wheat plus 2.5 g crushed wheat, and lids were fitted with a mesh screen lined on the outside with a thin layer of paper. Eggs of *P. interpunctella* (≈20 eggs/sample) were arranged onto strips of black filter paper with double sided sticky tape at one end of the strip to keep the eggs in place. The eggs were then placed into the vials of wheat, and vials were placed into a growth chamber and incubated at a constant temperature of 28ºC, 40-60% RH and a photoperiod of 16:8 h (L:D) for 14 days. Larval mortality of *P. interpunctella* was assessed on day 14 (see below).

Assessment of Mortality. Adult beetle mortality was assessed after the initial 7 days of incubation. The process involved separating the adult Coleoptera from the treated grain by sifting, then removing the insects all together and counting the live and dead adults. Two methods of removal were used depending on the apparent state of the insect's health: alive and healthy insects were removed with an aspirator, counted and disposed of; insects that appeared to be dying (moribund) or dead (not moving) were gently placed

into a Petri dish with a moist piece of filter paper and put back inside the growth chamber for 1 additional day. The next day beetles in the Petri dishes were checked for either living (moving live + moribund beetles) or dead adults, so a complete mortality assessment could be made for each treatment at each sample time. Mortality was expressed on a percentage basis from the original 50 insects used in each bioassay jar.

Larval mortality of *P. interpunctella* was assessed 14 days after bioassay vials were placed into the growth chamber. Eggs that had not hatched and appeared shriveled and dried up were not included in the mortality assessment, nor were newly hatched 1st instar larva that became stuck to the tape and died. Therefore the number of larva considered to be alive at the start of incubation were calculated by subtracting the number of stuck larva plus unhatched eggs from the total number of eggs initially arranged onto the filter paper. The number of larvae alive on day 14 was determined by emptying and separating apart the grain sample, some vials of which were tied together with larval silk, and counting the live larvae present. Mortality was then calculated by subtracting the number of live larva on day 14 from the number of live larva at the start of incubation, and was represented as a percentage.

Progeny Production. Assessment of progeny production of coleopteran species was conducted following the additional 49 day incubation period. Adult insects were sifted from grain samples, and then counted.

Data Analyses. Separate analyses by species were conducted for parent adult beetle mortality, adult beetle progeny, and larval mortality of *P. interpunctella*. Analyses of data were performed using a one-way analysis of variance (ANOVA) to determine differences due to treatments within each species and sample time. Means were then

separated for comparison using a protected least significant difference (LSD) test with significance at $P \leq 0.05$ (SAS Institute 2002).

Results

Mortality. Mean percent adult mortality for adult *R. dominica* is shown in Table 2-1. There was no statistical difference observe between different Spinosad formulation treatments at any sample time, and all Spinosad treatments achieved >99% killing efficacy throughout the duration of the study. Positive controls produced significantly lower mortalities than Spinosad formulations at all sample times in *R. dominica* bioassays. Results of mean percent adult mortality for *S. oryzae* are represented in Table 2-2. Spinosad liquid had very low mortality against *S. oryzae* adults at times 0, 32 and 48 weeks, but mortality at 16 weeks post treatment was substantially higher, at over 71%. However, control mortality of *S. oryzae* was also high at 16 weeks (58.3%) and not significantly different from that for Spinosad liquid. The Spinosad dust formulation produced a mortality rate against adult *S. oryzae* of consistently >90% at all sample times. The Spinosad Dry formulation, though less effective than the dust formulation at times 32 and 48 weeks, caused significantly high mortality rates at all sample times and was on a par with the activity of Spinosad dust at time 16 weeks. Complete mortality was produced by the positive controls at times 0 and 16 weeks, but by time 48 weeks mortality had dropped to ≈3%. Results for *T. castaneum* bioassays, shown in Table 2-3, presented no statistical difference between any insecticide treatment and the water only control at the 0 weeks post treatment sample time. A marked rise in adult mortality was

observed for the Spinosad dust treatment after the 0 weeks post treatment sample time, increasing >65% between the 0 and 16 weeks post treatment sample times, and maintaining a significantly higher killing efficacy than other treatments for the remainder of the study. Significant differences were not observed between other treatments and the water only control at the remaining sample times for *T. castaneum*.

Results for *P. interpunctella* larval mortality are presented in Table 2-4. The Spinosad dust formulation killed 100% of larvae at all sample times, and efficacies of the Spinosad Dry formulation were lower, but statistically similar to Spinosad dust at each sample time. The Spinosad liquid formulation showed a significantly higher percent mortality than the water only control at all sample times except at 0 weeks post treatment when the lowest larval mortality was observed for the liquid. The liquid formulation had a lower efficacy than the Storcide 2 positive control at all but the 48 weeks post treatment sample time, though the positive control's efficacy had declined by that point in time.

Progeny Production. *R. dominica* progeny production results are shown in Table 2-1. All insecticide treatments showed a significantly lower yield of progeny than did the water only control, and no difference was witnessed among insecticide treatments at any sample time, with progeny production averaging from 0 to 39.5 individuals among insecticide treatments, with these higher progeny numbers occurring only at time 48 weeks. Progeny counts for *S. oryzae* (Table 2-2) show a significantly lower progeny population for the Spinosad dust formulation than the other insecticide treatments at 32 and 48 week post treatment sample times. Progeny counts for all treatments, including the water only control, were not statistically different from one another at the 16 week post treatment sample time, and most progeny counts at this time were exceptionally low.

Differences between the dry and liquid Spinosad formulations were observed only at the 0 week sample time, in which progeny production in the dry Spinosad treated wheat was less than half that for Spinosad liquid, but at all other sample times the two treatments had similar results. *S. oryzae* progeny production peaked at the 48 week sample time for all treatments. Results for *T. castaneum* progeny counts (Table 2-3) showed the Spinosad liquid formulation to be significantly lower than the water only control at all sample times except for week 32. The dry and dust Spinosad formulations yielded similar results throughout the study, providing the greatest efficacy by keeping average progeny populations below 20 individuals per jar. The positive control provided similar results as the dry and dust Spinosad formulations at all but the 48 week sample time when population counts for the positive control were higher than the dry or dust, but still lower than the liquid Spinosad formulation and the water control.

Discussion

Several studies have shown a near complete mortality of *R. dominica* when exposed to Spinosad. Adult mortalities of 100% after 7 days of exposure have been reported with application rates as low as 0.1 mg/kg (Fang et al. 2002a), and LD_{50} values have been shown to occur at rates as low as 0.005 mg/kg (Huang et al. 2004). This high susceptibility has been documented in other species within the family Bostrichidae (Mutambuki et al. 2002), and it is not surprising therefore that such high adult mortality for *R. dominica* has been achieved in this study with all three formulations of Spinosad. High reductions in *R. dominica* progeny production have been reported with Spinosad

application rates of 0.1 mg/kg (Daglish 2006), which resemble results found in the current study.

Fang et. al (2002a) reported 7 day adult mortalities of *S. oryzae* exposed to wheat treated with 1 mg/kg of Spinosad between 69-100% depending on the wheat variety, and a mortality of ≈70% on hard red winter wheat. Another study showed 7-day adult mortalities wheat treated with the same application to be between 19-66% depending on strain of *S. oryzae* used (Nayak et al. 2005). Results from the current experiment did vary, though not due to a difference in strain or wheat variety, which were not tested. The dust formulation showed a high efficacy throughout the study, which supports findings from other studies describing Spinosad's ability to provide long term efficacy with low residual loss of activity in the absence of sunlight (Fang et al. 2002b, Subramanyam et al. 2002, Daglish et al. 2006). It was surprising that the dry formulation showed an apparent drop in efficacy between the 0 and 32 week sample times, suggesting that some sort of degradation may have occurred, though it would not have been due to photo-degradation since all treated grain was stored away from sunlight. All *S. oryzae* data reported here for the sample time of 16 weeks is potentially invalid as the adult mortality for the water control wheat was very high, which suggests some unknown experimental error was operating and masking the treatment effects. Nevertheless, the Spinosad dry formulation showed a consistent drop in activity at sample times 32 and 48 weeks, suggesting a degradation of the active ingredients at those times, perhaps due to the storage time in extreme temperature conditions. The liquid formulation showed very low efficacy at all sample times, suggesting that the particular formulation used was ineffective against the laboratory population of adult *S. oryzae* used in these bioassays. Progeny counts,

however, suggest that the liquid formulation did provide some control comparable to that of the dry formulation at 32 and 48 week sample times. Progeny counts also suggested the dust formulation of Spinosad to be the most effective of all the treatments over the duration of the study. Because *S. oryzae* inserts eggs into grain kernels and larvae develop totally within the kernel (Arbogast 1991), efficacy of a grain protectant against *S. oryzae* will many times be dependent on how many adult parents can be killed in a given time before they successfully mate and reproduce because eggs and larvae can escape exposure to many residual insecticides once inside a grain kernel. Dry and dust formulations of Spinosad had relative good activity against adult *S. oryzae* in these bioassays, with resulting low progeny production, but Spinosad liquid had low adult activity and thus allowed for substantial numbers of progeny to be produced.

The cause of the increase in mortality of adult *T. castaneum* in wheat treated with the Spinosad dust formulation between 0 and 16 week sample times is not known. Overall the dust formulation provided the best control out of the other treatments, although *T. castaneum* adults were the least susceptible of the tested species to any of the insecticide treatments. Such a low susceptibility of *T. castaneum* to Spinosad is a common trend among insecticidal studies on this topic (Mutambuki 2002, Toews and Subramanyam 2003, Toews et al. 2003, Huang et al. 2004), and in a more general point *Tribolium* species commonly show resistance to insecticidal control (Zettler 1991). Despite the low susceptibility of adult *T. castaneum*, the dry and dust formulations of Spinosad did effectively reduce progeny production, which is likely attributed to the newly hatched larvae being external feeders and free-living within the food media where

they readily contact the Spinosad, and due to larvae apparently having considerably higher susceptibility than adults to Spinosad (Huang et al. 2004).

Spinosad applied at 1 mg/kg has been shown to be highly effective in studies involving *P*. *interpunctella*, with larval mortalities ranging between 97-100% (Fang et al. 2002a). Similar results were found in this study with the dust formulation, and the dry formulation showed statistically similar outcomes, though results found with the liquid formulation once again suggest either an inadequacy in the formulation or the possibility of some degradation in the liquid Spinosad used, prior to this study.

In summary, the work reported here indicates and confirms that different formulations of Spinosad applied at a rate of 1 mg/kg may provide different levels of control depending on the pest species. Additionally, dry formulations of Spinosad, as opposed to liquid, and specifically the formulation referred to here as Spinosad dust, show promise as effective grain protectants on wheat that is threatened with infestation of the species studied here.

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Table 2-1. Adult mortality and progeny production of *R. dominica* exposed to specified treatments on wheat in bioassays conducted at different times after treatment.*

Sample Time (Post Treatment)	Treatment	% Mortality (± SE) 7 day exposure			Progeny (± SE) 49 day exposure		
0 weeks	Control	1.97	(1.39)	C	230.25	(37.68)	A
	Spinosad Liquid	100.00	(0.00)	A	4.25	(3.28)	B
	Spinosad Dry	100.00	(0.00)	A	0.00	(0.00)	B
	Spinosad Dust	100.00	(0.00)	A	0.00	(0.00)	B
	Storcide 2	89.42	(5.11)	B	0.25	(0.25)	B
16 weeks	Control	2.04	(1.18)	C	149.00	(56.24)	A
	Spinosad Liquid	100.00	(0.00)	A	1.25	(0.63)	B
	Spinosad Dry	99.02	(0.57)	A	0.25	(0.25)	B
	Spinosad Dust	100.00	(0.00)	A	0.25	(0.25)	B
	Storcide 2	56.41	(9.84)	B	0.50	(0.50)	B
32 weeks	Control	10.51	(4.42)	C	159.50	(27.92)	A
	Spinosad Liquid	100.00	(0.00)	A	3.00	(1.73)	B
	Spinosad Dry	100.00	(0.00)	A	0.00	(0.00)	B
	Spinosad Dust	100.00	(0.00)	A	0.00	(0.00)	B
	Storcide 2	76.48	(8.76)	B	0.75	(0.48)	B
48 weeks	Control	1.60	(1.04)	C	519.75	(88.40)	A
	Spinosad Liquid	99.49	(0.51)	A	22.25	(5.20)	B
	Spinosad Dry	100.00	(0.00)	A	0.25	(0.25)	B
	Spinosad Dust	100.00	(0.00)	A	0.00	(0.00)	B
	Storcide 2	23.50	(6.65)	B	39.50	(17.23)	B

*Means within a sample time group followed by the same letter are not statistically different (ANOVA and LSD test, P=0.05, n=4).

Table 2-2. Adult mortality and progeny production of *S. oryzae* exposed to specified treatments on wheat in bioassays conducted at different times after treatment.*

Sample Time (Post Treatment)	Treatment	% Mortality (± SE) 7 day exposure			Progeny (± SE) 49 day exposure		
0 weeks	Control	1.96	(1.96)	C	474.75	(42.91)	A
	Spinosad Liquid	1.52	(0.51)	C	395.25	(58.62)	A
	Spinosad Dry	91.96	(4.07)	B	137.25	(19.88)	B
	Spinosad Dust	99.49	(0.51)	A	39.75	(20.78)	BC
	Storcide 2	100.00	(0.00)	A	0.00	(0.00)	C
16 weeks	Control	58.30	(2.52)	B	13.00	(6.94)	A
	Spinosad Liquid	71.16	(14.24)	B	20.00	(19.34)	A
	Spinosad Dry	98.00	(1.41)	A	6.75	(2.50)	A
	Spinosad Dust	100.00	(0.00)	A	2.25	(2.25)	A
	Storcide 2	100.00	(0.00)	A	0.25	(0.25)	A
32 weeks	Control	0.51	(0.51)	C	354.00	(48.40)	A
	Spinosad Liquid	9.01	(4.72)	C	174.25	(21.25)	BC
	Spinosad Dry	52.08	(3.87)	B	217.50	(21.82)	B
	Spinosad Dust	98.98	(1.02)	A	13.75	(6.30)	D
	Storcide 2	58.13	(14.30)	B	109.25	(24.04)	C
48 weeks	Control	1.00	(1.00)	C	860.75	(77.02)	A
	Spinosad Liquid	2.01	(0.82)	C	668.50	(20.76)	B
	Spinosad Dry	55.13	(9.19)	B	537.75	(90.76)	B
	Spinosad Dust	91.00	(3.11)	A	165.75	(36.31)	C
	Storcide 2	2.94	(1.25)	C	575.00	(34.05)	B

*Means within a sample time group followed by the same letter are not statistically different (ANOVA and LSD test, P=0.05, n=4).

Table 2-3. Adult mortality and progeny production of *T. castaneum* exposed to specified treatments on wheat in bioassays conducted at different times after treatment.*

Sample Time (Post Treatment)	Treatment	% Mortality (± SE) 7 day exposure			Progeny (± SE) 49 day exposure		
0 weeks	Control	0.00	(0.00)	B *	192.25	(15.59)	A
	Spinosad Liquid	3.52	(2.21)	AB *	159.00	(13.89)	B
	Spinosad Dry	1.00	(1.00)	B *	4.00	(2.71)	C
	Spinosad Dust	5.69	(1.54)	A *	1.75	(0.48)	C
	Storcide 2	1.69	(1.69)	AB *	0.00	(0.00)	C
16 weeks	Control	3.10	(0.59)	B	134.75	(14.59)	A
	Spinosad Liquid	6.43	(3.30)	B	94.75	(11.48)	B
	Spinosad Dry	7.02	(4.43)	B	0.25	(0.25)	C
	Spinosad Dust	71.87	(10.52)	A	0.50	(0.50)	C
	Storcide 2	4.17	(2.55)	B	0.75	(0.48)	C
32 weeks	Control	1.00	(1.00)	B	64.50	(14.79)	A
	Spinosad Liquid	0.48	(0.48)	B	65.50	(5.72)	A
	Spinosad Dry	0.50	(0.50)	B	0.00	(0.00)	B
	Spinosad Dust	25.87	(8.59)	A	0.00	(0.00)	B
	Storcide 2	0.50	(0.50)	B	0.50	(0.50)	B
48 weeks	Control	0.00	(0.00)	B	303.00	(9.83)	A
	Spinosad Liquid	0.00	(0.00)	B	247.00	(13.80)	B
	Spinosad Dry	0.49	(0.49)	B	18.75	(8.78)	D
	Spinosad Dust	10.72	(3.30)	A	0.00	(0.00)	D
	Storcide 2	0.49	(0.49)	B	194.00	(25.75)	C

*Means within a sample time group followed by the same letter are not statistically different (ANOVA and LSD test, P=0.05, n=4).

Table 2-4. Mean % mortality of *P. interpunctella* larvae exposed for 14 days to specified treatments on wheat in bioassays conducted at different times after treatment.*

Sample Time (Post Treatment)	Treatment	% Mortality (± SE)		
0 weeks	Control	4.41	(4.41)	C
	Spinosad Liquid	13.27	(2.62)	C
	Spinosad Dry	88.06	(6.54)	A
	Spinosad Dust	100.00	(0.00)	A
	Storcide 2	73.06	(6.44)	B
16 weeks	Control	7.73	(2.89)	C
	Spinosad Liquid	53.50	(11.18)	B
	Spinosad Dry	96.88	(3.13)	A
	Spinosad Dust	100.00	(0.00)	A
	Storcide 2	91.34	(5.61)	A
32 weeks	Control	9.55	(2.87)	C
	Spinosad Liquid	54.72	(9.47)	B
	Spinosad Dry	98.61	(1.39)	A
	Spinosad Dust	100.00	(0.00)	A
	Storcide 2	89.93	(6.75)	A
48 weeks	Control	3.06	(1.78)	C
	Spinosad Liquid	32.14	(4.76)	B
	Spinosad Dry	100.00	(0.00)	A
	Spinosad Dust	100.00	(0.00)	A
	Storcide 2	34.55	(9.51)	B

*Means within a sample time group followed by the same letter are not statistically different (ANOVA and LSD test, P=0.05, n=4).

CHAPTER III

COMPARISONS OF EFFICACIES OF SPINOSAD AND CHLORPYRIFOS-METHYL APPLIED INDIVIDUALLY OR IN COMBINATION AGAINST STORED GRAIN INSECTS

Introduction

Reldan®, which has the active ingredient (AI) chlorpyriphos-methyl (c-m), was used in the United States as a stored grain residual protectant insecticide prior to its registration cancellation in 2007 (U.S. EPA 2007). Evaluations of the efficacy of Spinosad to control insect populations in stored grain have concluded that Spinosad can replace Reldan®, and other organophosphorous residual insecticides, for use on stored grain (Subramanyam et al. 2002). Use of Reldan® on stored grain is not banned worldwide, and the occurrence of insects that are resistant to c-m has been documented in several stored grain pests (Subramanyam et al. 1989, Zettler and Cuperus 1990, Zettler 1991, Subramanyam and Hagstrum 1995).

Delaying the occurrence of resistance to insecticides is an important part of pest management, and the use of various pesticides is a technique commonly used (Subramanyam and Hagstrum 1995). Several methods of multiple insecticide applications exist, and using two insecticides in combination as a mixture is the most effective approach, according to Subramanyam and Hagstrum (1995). However, inappropriate applications of mixtures could lead to a rapid development of resistance and it is therefore important to understand the outcome of treating pest outbreaks with such mixtures before it is adopted as a practical approach (Riddles and Nolan 1987).

Combination studies involving stored grain treated with Spinosad and c-m have been performed recently (Phillips and Bonjour personal communication, Nayak and

Daglish 2007). Phillips and Bonjour found that using the combination eventually caused a decrease in activity of Spinosad against adults of *Cryptolestes ferrugineus*, *Oryzaephilus surinamensis*, and *Sitophilus oryzae* as compared to treatments containing Spinosad alone at the same application rate as in the combination. Possible interactions between two multiple insecticides have been mentioned in other studies (Desmarchelier 1977, Daglish 1998), and the possibility that mixing Spinosad and c-m could result in a chemical antagonism is suggested by the earlier work. The following studies were performed to investigate this possibility further by:

1. performing bioassays on species of stored grain insects not previously tested to determine if the antagonistic effect occurs on other species;
2. exposing previously tested species to combinations of insecticides in ratios and combination patterns previously untested to determine if the antagonistic effect occurs at these different ratios;
3. analyzing chemical residue concentrations of the AI of Reldan (c-m) and the AIs of Spinosad (spinosyns A and D) at different ratios and at different times after application to determine if the antagonism is due to chemical degradation of one or more of the AIs.

Materials and Methods

Two separate experiments were set up to study the antagonistic effects previously observed when combining c-m and Spinosad. Hard red winter wheat that had no prior insecticide treatment was used in both experiments. Two thousand (2000) grams of

wheat were treated and housed inside 3.8 l glass jars. Methods for treatment of wheat were the same as reported in Chapter 2, in which treatments were applied to the inner surface of each jar, the wheat was added, and jars were rotated 20 full times initially and at intervals until all visible residues were removed from the inside surfaces of the jars, and a final 40 rotations was included to insure chemical treatments and wheat were mixed thoroughly (specific treatments are described below). Closed jars of the treated wheat were stored in a growth chamber in total darkness at a constant temperature of ≈ 28ºC.

Experiment 1.

Treatments. Treatments applied to the 3.8 l jars for the first experiment included a water only control, a formulation of c-m containing 43.2% AI in concentrate (Reldan 4E; Dow AgroSciences, Indianapolis, IN), a Spinosad formulation containing 22.8% AI in concentrate (SpinTor 2SC, Dow AgroSciences, Indianapolis, IN)), and a combination of c-m and Spinosad formulations. Five replications were made of each treatment, and target rates of application were as follows: c-m alone was applied at a rate of 6 mg of AI per kg of wheat (or 6 ppm), Spinosad alone was applied at a rate of 1 ppm, and the application rate of the combination was c-m at 3 ppm plus Spinosad at 1 ppm. The combination treatment was applied as a tank mix, in which the pesticides were mixed together with water in a glass beaker and applied as a mixture to the jar with wheat. It is important to mention that the plastic bottle that contained the commercial c-m concentrate mixed for the bioassays (see below) was cracked near the pouring spout for an unknown period of time prior to this study, and the quality of the c-m was unknown as it may have suffered oxidation or other change from exposure to air. Crystals were

observed in the container, which may have been evidence of a physical or chemical change to the product.

Bioassays. Wheat samples were taken from the treated 3.8 l jars at 1 week post-treatment, 12 weeks post-treatment, and 24 weeks post-treatment to conduct bioassays for pest beetle species *Lasioderma serricorne* (Anobiidae), *Tribolium confusum* (Tenebrionidae), *Cryptolestes ferrugineus* (Cucujidae), *Sitophilus oryzae* (Curculionidae), and *Oryzaephilus surinamensis* (Silvanidae). Bioassays were made using 237 ml glass jars, and placing 100g of treated wheat within each. Whole grain kernels of wheat were used for *S. oryzae*, and for the remaining species 5g of crushed wheat plus 95g whole wheat were used. Screw-on canning jar rings with a piece of copper mesh screen between two pieces of 7.0 cm filter paper were used as lids for the bioassay jars, and fifty adults of each species were added to individual jars. Bioassays were then stored in a growth chamber for 7 days at a constant temperature of 28ºC, a relative humidity of approximately 40-60% and a photoperiod of 16:8 h (L:D). After 7 days the adult beetles were removed to examine adult mortality, and the wheat and fine material were replaced into the 237 ml jars that were returned to the growth chamber for another 49 days to allow for progeny development and adult emergence within treated samples. All steps were performed at the 1, 12, and 24 week post treatment sample times.

Assessment of Mortality. Adult insects were sifted from wheat on the seventh day following bioassay set-up. Adults were separated from the wheat by sifting with metal screen sieves, and were counted and categorized as live, dead, or moribund. Live insects were removed and discarded. Dead and moribund insects were placed in a Petri dish

containing moist filter paper, stored in the growth chamber for an additional 24 hours to allow for insect recovery from treatment exposure, and then recounted to determine the final amount of live and dead insects. Counts of insects found to be living after the 24-hour recovery period were added to those determined as alive when insects were initially separated from the wheat. Mortality data for each treatment at each sample time were recorded as percentages of the original fifty adults added to each jar.

Progeny Production. Forty-nine days following the removal of parent adults and replacement of bioassays into the growth chambers, wheat samples were sifted once again to separate and count the number of adult insects in each sample.

Data Analysis. Data was analyzed separately for each species and treatment within each sample time. Adult mortality was analyzed using the analysis of variance (ANOVA) procedure, and analysis of progeny production was done using the General Linear Models (GLM) procedure (SAS Institute 2003). Means were compared using a least significant difference (LSD) test, and differences among means were determined as significant at $P \leq 0.05$.

Experiment 2.

Treatments. The experiment was conducted to examine the biological effects of different types of mixtures and the chemical fate of active ingredients following different mixture methods. Insecticides that were used in this experiment included Spinosad formulation SpinTor 2SC, and a newly formulated batch of Reldan® provided by Dow AgroSciences (DAS) after commercial lots of the product were not available for use. Target application rates for treatments include; Spinosad at 1 ppm, c-m at 3 ppm, c-m at 6 ppm, c-m at 3 ppm plus Spinosad at 1 ppm applied as a tank mix (3/1 tank), c-m at 6 ppm

plus Spinosad at 1 ppm applied as a tank mix (6/1 tank), c-m at 6 ppm plus Spinosad at 1 ppm applied sequentially (first c-m was applied and thoroughly mixed with 2000 g of wheat followed by the application and mixing of Spinosad in the same way, termed 6/1 sequential), and a water only control in which 1.5 ml of distilled water was applied. Five replications were set up for each treatment.

Assessment of Mortality. Pest species *C. ferrugineus* and *O. surinamensis* were used to assess adult mortality. To prepare the bioassays, 100 g samples of treated wheat were taken from the 3.8 l jars, and placed into 235 ml glass jars with 5 g of wheat from each sample crushed, and 95 g whole wheat. Samples were taken from the large jars at 4 different times; 0 weeks post-treatment (taken within 24 h after treatment), 12 weeks post-treatment, 24 weeks post-treatment, and 43 weeks post-treatment. Lids for bioassays were prepared in the same manner as Experiment 1. Fifty adults were placed into the 235 ml jars with each jar containing only one species. Bioassays were then placed into a growth chamber at a constant temperature of 28ºC 40-60% RH with a photoperiod of 16:8 h (L:D) for 7 days, at which point adult mortality was assessed. Adult mortality assessment was performed in the same manner as in Experiment 1; first categorizing insects as live, moribund, or dead; disposing of the live insects while placing moribund and apparently dead adults in a Petri dish with moist filter paper; placing the Petri dishes of moribund and dead insects back inside the growth chamber for 24 h; and then counting the remaining live and dead insects to include in the mortality assessment.

Insecticide Residue Analyses. Wheat samples were collected from the treated 3.8 l jars from Experiment 2 at 0 weeks post-treatment, 12 weeks post treatment, and 24 weeks post treatment. For each sample, 100 g of treated wheat were placed inside of two 940

ml zip-loc baggies. Replications of the same treatment were then placed inside one 3.8 l freezer bags, and the freezer bags were then stored inside of a freezer at –20ºC for overnight/weekend storage. Freezer bags were then placed into a Styrofoam ice chest with ice packs to keep them cold, and were sent by overnight delivery to DAS after each sample time so the amounts of residual AIs, specifically chlorpyrifos-methyl and Spinosyns A and D, could be analyzed from each sample to determine if there were losses residual AIs within each treatment that occurred between the times that the samples were taken. Quantitative chemical analyses of the AIs were performed by DAS using liquid chromatography tandem mass spectrometry (LC/MS/MS). Concentrations of chlorpyrifos-methyl and the sum total concentrations of the two Spinosyns, A+D, are reported as means per treatment and holding time.

Data Analysis. Adult mortality data were analyzed using the GLM procedure, and were analyzed separately for species and treatments within each sample time. Insecticide residue analysis data were processed using the ANOVA procedure, in which residues of AIs were processed separately within each sample time. Means for both data sets were subjected to separate LSD tests, and differences between means were determined at $P \leq 0.05$ level (SAS Institute 2002).

Results

Experiment 1.

Mortality. *L. serricorne* adult mortality (Table 3-1) was relatively high in the water only controls at all sample times, ranging between 19.83 and 23.37%. All treatments caused a

statistically higher mortality than the controls with the exception of Spinosad at 1 mg/kg in the 1-week post-treatment sample time. Spinosad caused greater mortality than controls at 12 and 24 weeks with a maximum mortality rate of 56.24%. The greatest mortality was caused by c-m at 6 mg/kg and by the combination of c-m and Spinosad; both treatments resulted in nearly complete adult mortality, with the combination causing between 98.8 and 100% mortality, and c-m alone causing between 99.6 and 100 % mortality.

Results of adult *T. confusum* mortality (Table 3-2) showed complete mortality from the c-m and the combination treatments at 1 week. Efficacies of both the c-m and combination treatments declined at each sample time, with the c-m treatment causing the greater mortality than other treatments at 12 weeks, 93.88% mortality, but only 19.62% mortality at 24 weeks. The efficacy of the combination treatment was much less at 12 weeks than earlier, causing a mortality of 29.05%. Spinosad caused a low mortality at all sample times, which were lower than 3% at all cases. Tests of significant differences were not made at 24 weeks because the experiment-wise treatment effect was not significant ($P>0.05$).

Adult mortality of *C. ferrugineus* (Table 3-3) showed a marked decrease in efficacy of the combination treatment between 12 and 24 weeks, dropping from 96% in week 12 to 24.65% in week 24. The efficacy of the Spinosad only treatment, however, never dropped below 98% at any sample time. The c-m treatment caused complete mortality at 1 and 12 weeks, and efficacy was statistically similar to Spinosad at those times. However, c-m then lost efficacy against *C. ferrugineus* adults by week 24 with a lower mortality of 88.25%, which was lower than Spinosad alone.

Both the c-m treatment and the combination treatment caused complete mortality in adult *S. oryzae* at all sample times (Table 3-4). Mortalities in Spinosad treated samples were statistically lower than those for c-m and combination treatments at the 1 and 12 week sample times, with mortality means between 82 and 91%. Spinosad treatments did show similar results to c-m alone and combination treatments for adult *S. oryzae* at the 24-week sample time, with a mortality of 98.82%.

O. surinamensis was highly susceptible to c-m treated wheat (Table 3-5) at 1 and 12 week sample times with adult mortalities >98%, but reduced activity was recorded for week 24 when mortality was ≈60%. Mortality in Spinosad treated samples was between 45 and 50% at all sample times, and mean mortalities were significantly lower than those for c-m treated samples at all times except for the 24 week sample, at which point mortality values were statistically similar. Samples containing the combination treatment showed complete mortality at the 1-week sample time, which was ≈50% higher than mortality in Spinosad treated samples at that time. Mortality in combination-treated samples was similar to that of Spinosad-treated samples at 12 weeks, and there was a mortality of 64.13% for c-m samples, but at the 24 week sample time the combination treatment produced a mortality of <10%, which was significantly lower than samples treated with Spinosad alone.

Progeny Production. Mean progeny counts of *L. serricorne* in all insecticide treated samples were significantly similar at all sample times, and were significantly lower than water only treatments (Table 3-1). c-m treated samples produced means between 0 and 1.4 individuals throughout the study, samples of Spinosad treatments showed mean progeny counts between 1.8 and 9 individuals, and progeny counts of combination

samples were between 0.2 and 0.4 individuals. A similar trend was observed in progeny counts of *T. confusum* (Table 3-2), with mean progeny counts of 0 or 0.2 for all insecticide treated samples at all times. Progeny counts for *C. ferrugineus* (Table 3-3) revealed no statistical differences among any of the insecticide treated samples at any sample time.

Differences in progeny counts among treatments did occur in bioassays with *S. oryzae* (Table 3-4). Spinosad treated samples yielded the highest number of progeny among the insecticide treated samples, in which average numbers of progeny were between 110.8 and 343.4 individuals. Chlorpyrifos-methyl treated samples had fewer than 10 individuals on average at any sample time, and means were statistically similar to those found in combination treated samples at 1 and 12 week sample times. Samples of combination treatments taken at 24 weeks did have a statistically higher mean number of progeny than did Reldan® treated samples at the same sample time with a mean count of 48.2 individuals in combination samples versus 9.6 in samples containing c-m alone.

Results for mean progeny counts of *O. surinamensis* are presented in Table 3-5. Mean progeny counts for c-m and combination treated samples were all low and statistically similar at all sample times. Progeny numbers in c-m treated samples averaged between 0 and 0.8, while samples with combination treatments averaged between 0 and 8.6 individuals. Results in samples containing Spinosad treatments were statistically similar to c-m and combination treated samples at 1 and 24 weeks. Progeny counts for Spinosad treated samples were statistically higher than the other insecticide treated samples at 12 weeks when progeny in Spinosad samples averaged at 164.8 individuals.

Experiment 2.

Mortality. Table 3-6 shows results of mean adult mortality observed in Experiment 2 for the *C. ferrugineus*. All insecticide treatments at 0 and 12 week sample times produced statistically similar results, and all caused complete mortality at those times except for the Spinosad at 1 ppm treatment at 0 and 12 weeks and c-m at 3 ppm at 12 weeks, in which cases both treatments produced mortalities >99%. c-m at 3 ppm produced the lowest efficacy of the insecticide treated samples at the 24-week sample time yielding an adult mortality of 81.49%, meanwhile all other insecticide treated samples remained statistically similar with means ranging between 91 and 100%. Results at this 24-week sample time were expected to show a lower efficacy in combination treatments than one or more treatments containing only Spinosad or only c-m, similar to results found in experiment 1, though such results did not occur at this sample time. However, at the 43-week sample time, when Spinosad at 1 ppm had very high mortality (99.58%), the 3/1-tank combination treatment of c-m with Spinosad produced a significantly lower mortality of 88.26%. The c-m at 3 ppm treatment had the lowest activity of all insecticide treatments at 43 weeks, but all insecticide treatments showed a higher mortality than the control. Both the 6/1-tank mix treatment, and the 6/1-sequential treatment produced results statistically similar to those for Spinosad alone at 1 ppm, and these also yielded higher mortalities than did the c-m at 6 ppm.

Bioassay results for *O. surinamensis* adults in Experiment 2 (Table 3-6) showed complete mortality for all treatments containing c-m at the 0-week sample time, and a

statistically lower mortality in the Spinosad treated samples, which averaged 82.2%. Mortalities from Spinosad at 1 ppm were also statistically lower than other insecticide treatments at the 12-week sample time. Mortality at the 24-week sample time from Spinosad at 1 ppm and c-m at 3 ppm were statistically similar. Mean mortality for c-m at 3 ppm at the 43-week sample time was statistically similar to that of the control, and were the lowest of the other insecticide treatments, followed by Spinosad at 1 ppm and the 3/1 tank treatment, which were statistically the same. Treatments containing c-m at 6 ppm remained the most effective treatments at the 12, 24, and 43-week sample times, though by the 43-week sample time the Spinosad combination treatments containing c-m at 6 ppm proved to be more effective than the c-m at 6 ppm treatment alone.

Insecticide Residue Analyses. Table 3-7 represents results of chemical analyses performed by DAS and statistical analyses processed thereafter. Chlorpyrifos-methyl residues in all samples treated with c-m at target application rate of 6 ppm, including both 6/1-combination treatments, were statistically similar at all sample times. Actual residue concentrations for all insecticide treatments fell below the target application rates. At the 12-week sample time, the mean AI concentrations for treatments of c-m applied at 3 ppm were lower than mean AI residuals in the 3/1 tank treatment, though at 0 and 24 weeks residual concentrations of the two treatments were statistically similar.

Spinosyn residues analyzed at the 0-week sample time showed only the 3/1 tank combination treatments had mean AI comparable to the Spinosad at 1 mg/kg treatment, while both 6/1 combination treatments had statistically lower AI residuals with the 6/1 tank combination treatments having the lowest amount of AI at 0.28 ppm. Spinosyn residues from grain treated with Spinosad at 1 ppm at the 12-week sample time were

higher than in all three combination treatments, with AI residues in the 6/1-tank treatment once again averaging lower than grain treated with Spinosad only. Spinosyn residues in all treatments receiving Spinosad application were statistically similar at the 24-week sample time with the lowest mean AI residuals found in the 6/1 tank mix at 0.35 ppm, and the highest mean AI residuals in Spinosad-only application of 1 ppm, yielding an average residue of 0.43 ppm.

Discussion

Relatively high adult mortalities of *L. serricorne* found in control bioassays in Experiment 1 after 7 days on treated grain were likely due to the short adult life span known for this species (Arbogast 1991). Adults of *L. serricorne* appear to be very fragile compared to adults of other beetle species used here, which may have augmented high adult mortalities. Complete control of *L. serricorne* using Spinosad has been reported at an application rate of 50 ppm on stored tobacco, though at 5 ppm complete development was still lower than 20% (Blanc et al. 2004). Results from Experiment 1 show a low efficacy for Spinosad on adult *L. serricorne* (Table 3-1) when applied at 1 ppm compared to treatments that had combined application with c-m. Nevertheless, suppression of progeny was nearly equal for all insecticide treatments, whether single or combined. This result could support the proposition that Spinosad's action against *L. serricorne* could be a cumulative process lasting the duration of the insect's life cycle (Blanc et al. 2004), or could be explained by decreased feeding found to occur in adults as compared to larvae (Ashworth 1993). Results reported here show that Spinosad has low activity

against *L. serricorne* adults and high activity against immature stages, and there was no impact of c-m on the activity of Spinosad for this species.

Complete control of adult *T. confusum* after 14 days of exposure, and suppression of progeny has been achieved with treatments of Spinosad applied to wheat at 1 ppm (Subramanyam et al. 2002). Experiment 1 showed very low adult mortality of *T. confusum* after 7 days, though progeny production was completely suppressed or held at a very low level (Table 3-2). *Tribolium confusum* resistance to chlorpyrifos-methyl has been documented in some strains, though mostly at low levels (Zettler 1991). Experiment 1 results appeared to show a decline in efficacy of c-m at ppm between the 1 and 24 week sample times, and complete control was achieved only at the 1 week sample time. The combination treatment appeared to follow a similar trend of chlorpyrifos-methyl degradation or loss of activity with storage time, though it was more drastic, probably due to a lower dosage of c-m, and at week 24 Spinosad at 1 ppm was statistically as effective as the combination. No negative affects on activity of either toxin from the combination treatment were seen in the *T. confusum* bioassay.

The potential for chemical interactions between two insecticides used in combination have been reported in past literature (e.g., Desmarchelier 1977, Daglish 1998). Prior studies involving the combination of c-m and Spinosad to control psocids showed no evidence of an interaction between the two insecticides (Nayak and Daglish 2007), while other combination studies showed that c-m combined with Spinosad is less effective in controlling adult mortality than Spinosad alone after a storage period of 12 and 24 weeks for the species *C. ferrugineus, S. oryzae,* and *O. surinamensis* (Phillips and Bonjour). Very similar results of an interaction effect were found in Experiment 1 with

C. ferrugineus (Table 3-3) and *O. surinamensis* (Table 3-5) after a storage period of 24 weeks, but the combination effect was not seen with *S. oryzae* (Table 3-4). The same combination effect was observed in Experiment 2 with *C. ferrugineus* (Table 3-6) to a lesser extent than in Experiment 1, although it was not evident until 43-weeks after application, and it only occurred with the 3/1-tank mix combination treatment. *O. surinamensis* did not show signs of a Spinosad efficacy decrease affect from a purported interaction as a result of using any of the three combinations at any sample time in Experiment 2. One possible reason for the differences in the combination effect between experiments was that for Experiment 2 a brand new, "hand-crafted" c-m was provided by DAS, and the formulation used in experiment 1 was older and was derived from a mass-produced commercial manufacturing run. Despite the age of the c-m used in Experiment 1, and the fact that loss of activity was suspected or predicted due to the discovery of a damaged holding container, high mortalities were observed in bioassays at 1-week, which suggest the older and potentially tainted c-m still retained much of its insecticidal activity. Observations reported by Lessard et al. (1993) support this assumption in which no survival in *S. oryzae* occurred in the first bioassays treated with c-m, and when compared to data in Experiment 1, complete mortality was observed with nearly all bioassays at 1 week. Results on F1 progeny production in Experiment 1 (Tables 3-1 through 3-5) did not show evidence of any interaction between AI in combination treatments, and instead provided equal or greater control than Spinosad alone for up to 24 weeks, which suggest that both Spinosad and c-m, whether in combination or singly, are active against immature stages of external grain feeding insects used in these bioassays, with no negative interactions. Furthermore, a treatment's ability to control numbers of

progeny is more than likely the most important factor of consideration when determining a treatments ability to control pest population outbreaks (Daglish 1998).

Analysis of the residue data provided by DAS (Table 3-7) in Experiment 2 showed no evidence of a reduction in c-m residues when in combination with Spinosad when compared to c-m residues in applications in which this AI was applied by itself. Data did clearly support of degradation of Spinosyns in combination applications with c-m at 0 weeks, when both 6/1 combinations showed statistically lower residues than the Spinosad only application at 1 ppm, and at 12 weeks when all three combination treatments showed lower Spinosyn residues than Spinosad at 1 ppm. However, data recorded from samples taken at 24 weeks were not consistent with those from 12 weeks as the degradation effect of c-m was not statistically supported when comparing the combination with the Spinosad only. The reason for this equivocal result may simply be due to sampling error, in which variance in residues was high and sample numbers low such that differences not due to the combination treatment were predicted to occur in more than 5% of the cases. The measured concentrations of the Spinosyns were numerically lower in the combination treatments when compared to the Spinosad only treatment at the same applied target rate.

In summary, it is possible that the newness of the c-m could determine the time it takes for any evidence of antagonism to occur. Insects not previously studied, *L. serricorne* and *T. confusum*, did not present any evidence that an antagonism had occurred. Of the different ratios of c-m mixed with Spinosad only the 3/1 combination showed any evidence of antagonism, and the exact nature of antagonism remains uncertain.

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Table 3-1. Adult mortality and progeny production of *L. serricorne* exposed to specified treatments on wheat in bioassays conducted at different times after treatment in Experiment 1.

Sample Time (Post Treatment)	Treatments c-m/spin (mg/kg)	% Mortality (± SE) 7 day exposure			Progeny (± SE) 49 day exposure		
1 week	0/0	19.83	(1.83)	B	206.60	(40.20)	A
	6/0	100.00	(0.00)	A	0.20	(0.20)	B
	0/1	27.40	(5.71)	B	1.80	(0.37)	B
	3/1	100.00	(0.00)	A	0.20	(0.20)	B
12 weeks	0/0	23.37	(3.33)	C	566.80	(62.58)	A
	6/0	100.00	(0.00)	A	0.00	(0.00)	B
	0/1	56.24	(2.02)	B	9.00	(2.59)	B
	3/1	100.00	(0.00)	A	0.40	(0.24)	B
24 weeks	0/0	23.15	(1.92)	C	653.60	(78.67)	A
	6/0	99.60	(0.40)	A	1.40	(0.40)	B
	0/1	35.51	(2.04)	B	7.40	(1.69)	B
	3/1	98.80	(0.80)	A	0.25	(0.25)	B

Means within a sample time group followed by the same letter are not statistically different (ANOVA/GLM and LSD test, $P<0.05$, n=4).

Table 3-2. Adult mortality and progeny production of *T. confusum* exposed to specified treatments on wheat in bioassays conducted at different times after treatment in Experiment 1.

Sample Time (Post Treatment)	Treatments c-m/spin (mg/kg)	% Mortality (± SE) 7 day exposure			Progeny (± SE) 49 day exposure		
1 week	0/0	0.40	(0.40)	C	31.00	(3.27)	A
	6/0	100.00	(0.00)	A	0.20	(0.20)	B
	0/1	2.80	(1.20)	B	0.00	(0.00)	B
	3/1	100.00	(0.00)	A	0.20	(0.20)	B
12 weeks	0/0	0.00	(0.00)	C	176.00	(23.95)	A
	6/0	93.88	(6.12)	A	0.20	(0.20)	B
	0/1	1.20	(1.20)	C	0.00	(0.00)	B
	3/1	29.05	(7.92)	B	0.00	(0.00)	B
24 weeks	0/0	0.00	(0.00)	A	165.80	(19.04)	A
	6/0	19.62	(10.39)	A	0.00	(0.00)	B
	0/1	2.42	(0.76)	A	0.20	(0.20)	B
	3/1	3.19	(1.02)	A	0.00	(0.00)	B

Means within a sample time group followed by the same letter are not statistically different (ANOVA/GLM and LSD test, $P<0.05$, n=4).

Table 3-3. Adult mortality and progeny production of *C. ferrugineus* exposed to specified treatments on wheat in bioassays conducted at different times after treatment in Experiment 1.

Sample Time (Post Treatment)	Treatments c-m/spin (mg/kg)	% Mortality (± SE) 7 day exposure			Progeny (± SE) 49 day exposure		
1 week	0/0	15.67	(4.20)	B	7.80	(2.48)	A
	6/0	100.00	(0.00)	A	0.00	(0.00)	B
	0/1	99.58	(0.42)	A	0.20	(0.20)	B
	3/1	100.00	(0.00)	A	0.40	(0.24)	B
12 weeks	0/0	1.62	(0.76)	B	269.00	(23.28)	A
	6/0	100.00	(0.00)	A	0.60	(0.60)	B
	0/1	99.19	(0.49)	A	0.40	(0.24)	B
	3/1	96.00	(3.03)	A	0.20	(0.20)	B
24 weeks	0/0	0.80	(0.49)	D	144.60	(5.32)	A
	6/0	88.25	(2.85)	B	0.60	(0.40)	B
	0/1	98.77	(0.82)	A	0.20	(0.20)	B
	3/1	24.65	(3.91)	C	0.40	(0.40)	B

Means within a sample time group followed by the same letter are not statistically different (ANOVA/GLM and LSD test, $P<0.05$, n=4).

Table 3-4. Adult mortality and progeny production of *S. oryzae* exposed to specified treatments on wheat in bioassays conducted at different times after treatment in Experiment 1.

Sample Time (Post Treatment)	Treatments c-m/spin (mg/kg)	% Mortality (± SE) 7 day exposure			Progeny (± SE) 49 day exposure		
1 week	0/0	1.27	(0.52)	C	461.20	(58.16)	A
	6/0	100.00	(0.00)	A	0.60	(0.40)	C
	0/1	90.80	(3.26)	B	174.80	(48.36)	B
	3/1	100.00	(0.00)	A	0.40	(0.24)	C
12 weeks	0/0	1.20	(0.49)	C	642.20	(49.10)	A
	6/0	100.00	(0.00)	A	0.00	(0.00)	C
	0/1	82.23	(4.96)	B	343.40	(51.70)	B
	3/1	100.00	(0.00)	A	11.60	(1.94)	C
24 weeks	0/0	1.59	(0.40)	B	346.60	(18.28)	A
	6/0	100.00	(0.00)	A	9.60	(4.17)	D
	0/1	98.82	(0.79)	A	110.80	(13.98)	B
	3/1	100.00	(0.00)	A	48.20	(7.12)	C

Means within a sample time group followed by the same letter are not statistically different (ANOVA/GLM and LSD test, $P<0.05$, n=4).

Table 3-5. Adult mortality and progeny production of *O. surinamensis* exposed to specified treatments on wheat in bioassays conducted at different times after treatment in Experiment 1.

Sample Time (Post Treatment)	Treatments c-m/spin (mg/kg)	% Mortality (± SE) 7 day exposure			Progeny (± SE) 49 day exposure		
1 week	0/0	7.64	(2.07)	C	225.60	(60.26)	A
	6/0	99.54	(0.47)	A	0.20	(0.20)	B
	0/1	49.21	(5.18)	B	3.60	(2.40)	B
	3/1	100.00	(0.00)	A	0.00	(0.00)	B
12 weeks	0/0	3.67	(1.65)	C	591.00	(36.62)	A
	6/0	98.79	(0.80)	A	0.00	(0.00)	C
	0/1	48.95	(7.38)	B	164.80	(34.81)	B
	3/1	64.13	(9.60)	B	3.40	(2.68)	C
24 weeks	0/0	0.39	(0.39)	B	583.60	(63.96)	A
	6/0	60.28	(8.10)	A	0.80	(0.80)	B
	0/1	45.27	(7.96)	A	56.40	(19.12)	B
	3/1	9.24	(2.21)	B	8.60	(2.87)	B

Means within a sample time group followed by the same letter are not statistically different (ANOVA/GLM and LSD test, $P<0.05$, n=4).

Table 3-6. Adult mortality of *C. ferrugineus* and *O. surinamensis* exposed to specified treatments on wheat in bioassays at different times after treatment in Experiment 2.

Sample Time (Post Treatment)	Treatment c-m/spin (mg/kg)	% Mortality (± SE) *C. ferrugineus*			% Mortality (± SE) *O.* surinamensis		
0 weeks	0/0	3.19	(1.02)	B	11.72	(3.75)	C
	0/1	99.18	(0.50)	A	82.20	(4.08)	B
	3/0	100.00	(0.00)	A	100.00	(0.00)	A
	6/0	100.00	(0.00)	A	100.00	(0.00)	A
	3/1 (tank mix)	100.00	(0.00)	A	100.00	(0.00)	A
	6/1 (tank mix)	100.00	(0.00)	A	100.00	(0.00)	A
	6/1 (sequential)	100.00	(0.00)	A	100.00	(0.00)	A
12 weeks	0/0	0.82	(0.50)	B	2.73	(1.44)	E
	0/1	99.38	(0.42)	A	52.75	(4.74)	D
	3/0	99.20	(0.80)	A	81.86	(4.82)	C
	6/0	100.00	(0.00)	A	100.00	(0.00)	A
	3/1 (tank mix)	100.00	(0.00)	A	89.98	(4.33)	BC
	6/1 (tank mix)	100.00	(0.00)	A	97.60	(1.60)	AB
	6/1 (sequential)	100.00	(0.00)	A	100.00	(0.00)	A
24 weeks	0/0	7.20	(1.74)	C	12.27	(3.08)	D
	0/1	98.38	(0.40)	A	47.57	(7.30)	C
	3/0	81.49	(7.69)	B	57.48	(15.84)	BC
	6/0	99.60	(0.40)	A	98.84	(0.77)	A
	3/1 (tank mix)	91.63	(2.23)	A	72.27	(10.35)	B
	6/1 (tank mix)	97.63	(1.57)	A	98.37	(1.00)	A
	6/1 (sequential)	100.00	(0.00)	A	99.20	(0.49)	A
43 weeks	0/0	1.59	(0.73)	E	1.63	(0.75)	D
	0/1	99.58	(0.42)	A	24.85	(5.13)	C
	3/0	15.68	(3.75)	D	4.08	(1.44)	D
	6/0	91.63	(5.08)	BC	52.22	(13.24)	B
	3/1 (tank mix)	88.26	(2.44)	C	29.07	(8.62)	C
	6/1 (tank mix)	98.78	(0.82)	AB	78.85	(3.92)	A
	6/1 (sequential)	100.00	(0.00)	A	89.36	(2.65)	A

Means within a sample time group followed by the same letter are not statistically different (GLM and LSD test, $P<0.05$, $n=5$).

Table 3-7. Residual amounts of active ingredients in specified treatments at different times after treatment in Experiment 2.

Sample Time Weeks Post-Treatment	Treatment-target application c-m/spin (mg/kg)	Actual Mean AI c-m (mg/kg)			Spinosyn A+D (mg/kg)		
0	0/0	0.00	(0,00)	C	0.00	(0.00)	D
	0/1	0.00	(0.00)	C	0.45	(0.01)	A
	3/0	2.18	(0.23)	B	0.00	(0.00)	D
	6/0	5.00	(0.51)	A	0.00	(0.00)	D
	3/1 (tank mix)	1.95	(0.11)	B	0.41	(0.03)	AB
	6/1 (tank mix)	4.14	(0.23)	A	0.28	(0.01)	C
	6/1 (sequential)	4.35	(0.50)	A	0.37	(0.04)	B
12	0/0	0.00	(0.00)	D	0.00	(0.00)	D
	0/1	0.00	(0.00)	D	0.56	(0.04)	A
	3/0	1.33	(0.14)	C	0.00	(0.00)	D
	6/0	4.69	(0.27)	A	0.00	(0.00)	D
	3/1 (tank mix)	2.01	(0.10)	B	0.45	(0.03)	B
	6/1 (tank mix)	4.37	(0.21)	A	0.30	(0.02)	C
	6/1 (sequential)	4.28	(0.32)	A	0.44	(0.04)	B
24	0/0	0.00	(0.00)	C	0.00	(0.00)	B
	0/1	0.00	(0.00)	C	0.43	(0.07)	A
	3/0	1.59	(0.09)	B	0.00	(0.00)	B
	6/0	3.27	(0.16)	A	0.00	(0.00)	B
	3/1 (tank mix)	1.30	(0.15)	B	0.36	(0.04)	A
	6/1 (tank mix)	3.23	(0.15)	A	0.35	(0.02)	A
	6/1 (sequential)	3.16	(0.07)	A	0.37	(0.02)	A

Means within a sample time group followed by the same letter are not statistically different (ANOVA and LSD test, $P<0.05$, $n=5$).

VITA

James Barrett Robertson

Candidate for the Degree of

Master of Science

Thesis: EFFICACY OF SPINOSAD FORMULATIONS TO CONTROL STORED GRAIN INSECTS

Major Field: Entomology

Biographical:

Personal Data: Born in Sapulpa, Oklahoma, On September 2, 1980, the son of James and Janie Robertson.

Education: Graduated from Follett High School, Follett, Texas in May 1999; received Bachelor of Science degree in Entomology from Oklahoma State University, Stillwater, Oklahoma in May 2005. Completed the requirements for the Master of Science degree with a major in Entomology at Oklahoma State University in December, 2008.

Experience: Worked as a farm hand near Slapout, Oklahoma, 1996 to 1999; employed by Oklahoma State University Physical Plant, Department of Truck Services, 2001 to 2004; employed by Oklahoma State University, Department of Entomology and Plant Pathology as a laboratory technician, 2004 to 2006; employed as a graduate research assistant by Oklahoma State University, Department of Entomology and Plant Pathology, 2006 through July 2008.

Professional Memberships: Sandborn Entomology Club.

Name: James Barrett Robertson Date of Degree: December, 2008

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of State: EFFICACY OF SPINOSAD FORMULTIONS TO CONTROL STORED GRAIN INSECTS

Pages in Study: 56 Candidate for the Degree of Master of Science

Major Field: Entomology

Scope and Method of Study: One study was conducted to determine if different formulations of Spinosad provide the same level of control against insect pests in stored wheat. Three different formulations of Spinosad were used, including 1 liquid formulation, a coarse dry formulation, and a dust formulation. Bioassays were conducted on the insect pest species *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium castaneum* and *Plodia interpunctella*, at different sample periods to determine efficacies of different Spinosad formulations. Another study was conducted to examine a possible chemical antagonism phenomenon that occurs when Spinosad is mixed with chlorpyrifos-methyl and applied to wheat, using treatments of Spinosad and chlorpyrifos-methyl applied individually, in different combinations, and with different methods of application. *Lasioderma serricorne*, *T. confusum*, *Cryptolestes ferrugineus*, *S. oryzae*, and *Oryzaephilus surinamensis* were among species used to study efficacies of different treatments. Chemical analyses were performed to examine degradations of treatment active ingredients (AI) over time.

Findings and conclusions: Differences in efficacies between two or more of the Spinosad formulations were observed in bioassays, depending on insect species tested. Results determined the liquid formulation was the least effective formulation of Spinosad in bioassays containing *S. oryzae*, *T. castaneum*, and *P. interpunctella*; and the dust formulation was most effective in bioassays containing *S. oryzae*, and *T. castaneum*. Evidence of antagonism was observed in wheat treated with a combination of chlorpyrifos-methyl applied at 3 mg/kg, plus Spinosad applied at 1 mg/kg in bioassays containing species *C. ferrugineus*, and *O. surinamensis*. Chemical analyses showed amounts of Spinosad AIs to be less when Spinosad is applied in combination treatments with chlorpyrifos-methyl, than when Spinosad is applied individually, though results were not conclusive enough to determine if chemical antagonism caused a reduction in Spinosad AIs.

Advisor's Approval:_____