

Speed of Action of Liquid and Dry Spinosad Formulations Applied to Whole Grains Against Stored-Product Beetles

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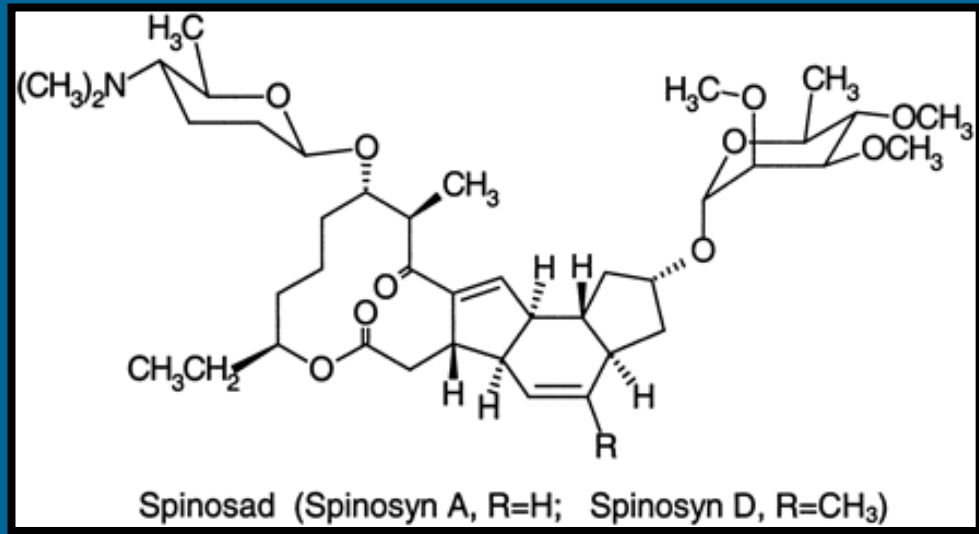
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Annual Meeting of the North Central Branch ESA

March 27, 2006

Spinosad

- ★ Insecticide derived from fermentation products (Spinosyns A and D) of the actinomycete bacterium, *Saccharopolyspora spinosa* (Mertz & Yao)
- ★ Unique mode of action reduces the chances of cross-resistance to traditional insecticides
- ★ Low mammalian toxicity
- ★ Environmentally benign
- ★ Registered by US-EPA in 2005 for use on grains
- ★ Commercially available in 2007, pending international tolerances



Previous Work

- ★ Spinosad has been tested on many crops and targets many pests (Sparks et. al 2001)
- ★ All previous published work with spinosad on stored-grain insects has been with a liquid formulation (SpinTor 2SC)
- ★ At 1 mg AI/kg (ppm) liquid spinosad killed and prevented population growth of key stored-grain insects under laboratory and field conditions (Fang et al. 2002a,b; Huang and Subramanyam 2003; Flinn et al. 2004)
- ★ Experiments have shown that at 1 mg AI/kg, dry spinosad controls rice weevil and lesser grain borer based on adult mortality and progeny suppression

Speed of Kill

- ★ Stored-product insect adults vary in susceptibility (Toews and Subramanyam, 2003)
- ★ Mortality is typically assessed 7-14 days after exposure
- ★ Some species may die quicker than others
- ★ Some species may recover or show delayed mortality

Acute mortality: proportion of insects that die, assessed immediately after exposure to spinosad

End-point mortality: proportion of insects that die, assessed 24 h after insects are transferred from treated grain to clean grain

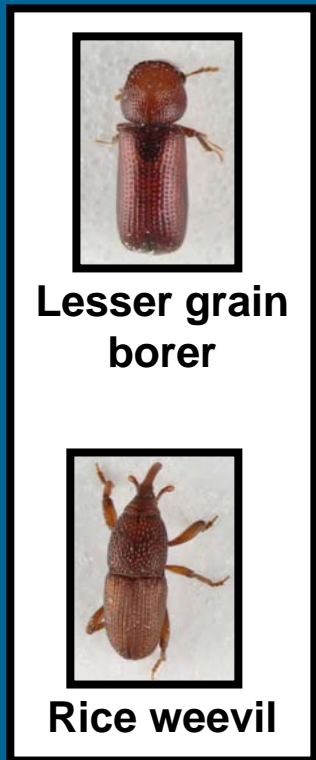
Objectives

1. Determine the lethal time to kill 50% of a population (LT_{50}) of stored-grain insects exposed to spinosad:
 - ★ On wheat, corn, and sorghum
 - ★ Using dry and liquid spinosad formulations at 1 mg (AI)/kg (the labeled rate)
 - ★ Assess acute and end-point mortality

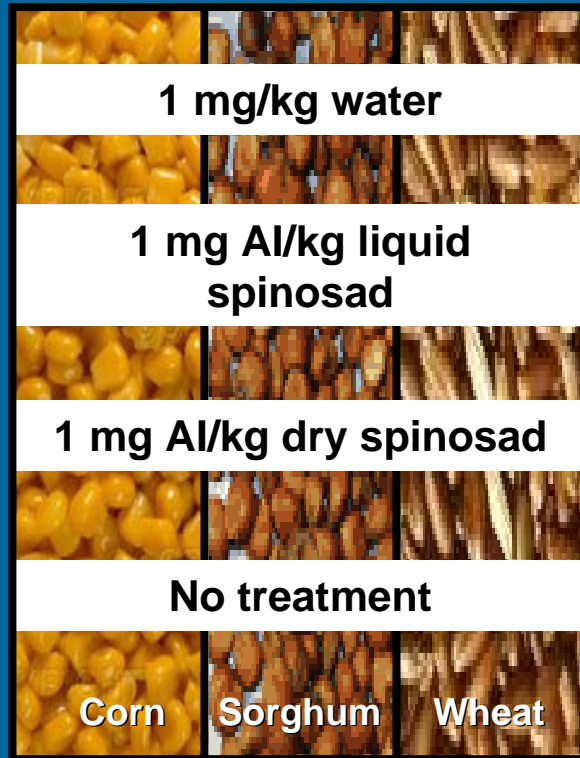
2. Pair-wise comparisons of LT_{50} values based on:
 - ★ Commodity
 - ★ Formulation
 - ★ Species
 - ★ Mortality assessment

Experimental Design

Treatments



25 mixed age unsexed adult insects



X 3 replicates



9-11 time intervals:
0.5 hours to 19 days

Acute mortality assessment



24 hours

End-point mortality assessment

Mortality Assessment

Acute:

- ★ After an exposure period to spinosad, insects were immediately sifted from the grain
- ★ The proportion of dead (knocked down) insects were determined
- ★ All insects were transferred to clean grain for end-point mortality assessment



End-point:

- ★ After 24 h on clean grain, insects were immediately sifted from grain
- ★ The proportion of dead insects were calculated

Statistical Analyses

LT₅₀s:

- ★ Data was corrected for mortality and subjected to probit analysis
- ★ The goodness-of-fit of the model was compared using a χ^2 statistic with significance at $\alpha = 0.05$

Pair-wise comparisons:

- ★ LT₅₀s were compared using lethal time ratios, as described by Robertson and Priesler (1992)
- ★ Comparisons were significantly different if the ratio confidence limits did not include 1

Lesser Grain Borer Time-Mortality Estimates

Commodity	Form.	Mortality Assessment	LT ₅₀ (95% CL) (days)	χ^2 (df)	P-value
Corn (750 insects)	Dry	Acute	0.522 (0.413-0.642)	12.849 (8)	0.1175
		End-point	0.044 (0.003-0.119)	16.913 (8)	0.0310*
	Liquid	Acute	0.743 (0.430-1.136)	19.899 (8)	0.0107*
		End-point	0.133 (0.076-0.197)	10.245 (8)	0.2483
Wheat (825 insects)	Dry	Acute	0.528 (0.248-0.922)	32.467 (9)	0.0002*
		End-point	0.053 (0.001-0.153)	66.638 (9)	0.0001*
	Liquid	Acute	0.476 (0.365-0.600)	10.369 (9)	0.3214
		End-point	0.059 (0.009-0.122)	33.762 (9)	0.0001*
Sorghum (750 insects)	Dry	Acute	0.447 (0.209-0.738)	26.816 (8)	0.0008*
		End-point	0.228 (0.163-0.293)	7.889 (8)	0.4444
	Liquid	Acute	0.451 (0.290-0.634)	14.366 (8)	0.0727
		End-point	0.223 (0.159-0.288)	9.850 (8)	0.2757

* Significant ($P < 0.05$)

Rice Weevil Time-Mortality Estimates

Commodity	Form.	Mortality Assessment	LT ₅₀ (95% CL) (days)	χ^2 (df)	P-value
Corn (750 insects)	Dry	Acute	3.295 (no CL)	37.511 (8)	0.0001*
		End-point	3.408 (no CL)	47.469 (8)	0.0001*
	Liquid	Acute	3.301 (2.336-4.190)	11.761 (8)	0.1622
		End-point	3.241 (2.242-4.155)	9.104 (8)	0.3336
Wheat (1125 insects)	Dry	Acute	3.267 (2.729-3.850)	7.516 (8)	0.4821
		End-point	2.747 (1.853-3.569)	14.118 (8)	0.0788
	Liquid	Acute	2.776 (1.684-3.645)	31.930 (7)	0.0001*
		End-point	2.062 (0.132-3.667)	93.037 (8)	0.0001*
Sorghum (1350 insects)	Dry	Acute	4.556 (2.965-5.944)	55.629 (9)	0.0001*
		End-point	4.517 (2.937-5.881)	53.551 (9)	0.0001*
	Liquid	Acute	3.460 (3.088-3.806)	10.836 (9)	0.1460
		End-point	3.432 (3.077-3.761)	11.686 (9)	0.1114

* Significant ($P < 0.05$)

Pair-wise Comparisons of Species

Commodity	Formulation	Mortality Assessment	Species ^{ab}	LT ₅₀ Ratio (95% CL)
Wheat	Dry	Acute	LGB vs <i>RW</i>	6.19 (3.65-10.5)*
		End-point	LGB vs <i>RW</i>	51.39 (13.66-193.28)*
	Liquid	Acute	LGB vs <i>RW</i>	5.83 (4.05-8.41)*
		End-point	LGB vs <i>RW</i>	34.78 (11.80-102.52)*
Sorghum	Dry	Acute	LGB vs <i>RW</i>	6.31 (2.42-16.47)*
		End-point	LGB vs <i>RW</i>	76.80 (16.06-387.15)*
	Liquid	Acute	LGB vs <i>RW</i>	4.45 (2.76-7.16)*
		End-point	LGB vs <i>RW</i>	24.36 (14.06-42.20)*
Corn	Dry	Acute	LGB vs <i>RW</i>	10.20 (6.00-17.34)*
		End-point	LGB vs <i>RW</i>	19.86 (13.61-28.98)*
	Liquid	Acute	LGB vs <i>RW</i>	7.69 (5.53-10.68)*
		End-point	LGB vs <i>RW</i>	15.39 (11.31-20.92)*

^a LGB, lesser grain borer; RW, rice weevil

^b Larger LT₅₀ written in *italics*

* Significantly different ($P < 0.05$)

Lesser Grain Borer Pair-wise Comparisons

Mortality
Assessment
Comparisons

Commodity	Formulation	Mortality Assessment ^a	LT ₅₀ Ratio (95% CL)
Wheat	Dry	<i>Acute</i> vs End-point	9.88 (2.45-39.75)*
	Liquid	<i>Acute</i> vs End-point	8.03 (3.33-19.37)*
Corn	Dry	<i>Acute</i> vs End-point	11.77 (3.47-39.94)*
	Liquid	<i>Acute</i> vs End-point	5.58 (3.06-10.18)*
Sorghum	Dry	<i>Acute</i> vs End-point	1.96 (1.13-3.41)*
	Liquid	<i>Acute</i> vs End-point	2.02 (1.32-3.09)*

Formulation
Comparisons

Commodity	Mortality Assessment	Formulation ^a	LT ₅₀ Ratio (95% CL)
Wheat	Acute	<i>Dry</i> vs Liquid	1.11 (0.63-1.94)
	End-point	Dry vs <i>Liquid</i>	1.11 (0.24-5.22)
Corn	Acute	Dry vs <i>Liquid</i>	1.42 (0.91-2.21)
	End-point	Dry vs <i>Liquid</i>	3.00 (0.83-10.87)
Sorghum	Acute	Dry vs <i>Liquid</i>	1.01 (0.57-1.77)
	End-point	<i>Dry</i> vs Liquid	1.02 (0.68-1.53)

^a Larger LT₅₀ written in *italics*

* Significantly different ($P < 0.05$)

Lesser Grain Borer Pair-wise Comparisons

Formulation	Mortality Assessment	Commodity ^a	LT ₅₀ Ratio (95% CL)
Dry	Acute	<i>Wheat vs Corn</i>	1.01 (0.58-1.75)
		<i>Wheat vs Sorghum</i>	1.18 (0.59-2.36)
		<i>Corn vs Sorghum</i>	1.17 (0.70-1.97)
	End-point	<i>Wheat vs Corn</i>	1.20 (0.21-7.07)
		<i>Wheat vs Sorghum</i>	4.26 (1.13-16.08)*
		<i>Corn vs Sorghum</i>	5.13 (1.49-17.65)*
Liquid	Acute	<i>Wheat vs Corn</i>	1.56 (0.99-2.46)
		<i>Wheat vs Sorghum</i>	1.06 (0.71-1.57)
		<i>Corn vs Sorghum</i>	1.65 (1.01-2.70)*
	End-point	<i>Wheat vs Corn</i>	2.24 (0.86-5.88)
		<i>Wheat vs Sorghum</i>	3.76 (1.54-9.20)
		<i>Corn vs Sorghum</i>	1.68 (0.97-2.90)

Commodity Comparisons

^a Larger LT₅₀ written in *italics*

* Significantly different ($P < 0.05$)

Rice Weevil Pair-wise Comparisons

Mortality
Assessment
Comparisons

Commodity	Formulation	Mortality Assessment ^a	LT ₅₀ Ratio (95% CL)
Wheat	Dry	<i>Acute vs End-point</i>	1.19 (0.87-1.62)
	Liquid	<i>Acute vs End-point</i>	1.35 (0.65-2.78)
Corn	Dry	<i>Acute vs End-point</i>	1.03 (0.26-4.07)
	Liquid	<i>Acute vs End-point</i>	1.02 (0.68-1.53)
Sorghum	Dry	<i>Acute vs End-point</i>	1.01 (0.71-1.42)
	Liquid	<i>Acute vs End-point</i>	1.01 (0.87-1.17)

Formulation
Comparisons

Commodity	Mortality Assessment	Formulation ^a	LT ₅₀ Ratio (95% CL)
Wheat	Acute	<i>Dry vs Liquid</i>	1.18 (0.86-1.61)
	End-point	<i>Dry vs Liquid</i>	1.33 (0.65-2.75)
Corn	Acute	<i>Dry vs Liquid</i>	1.00 (0.38-2.66)
	End-point	<i>Dry vs Liquid</i>	1.05 (0.37-2.99)
Sorghum	Acute	<i>Dry vs Liquid</i>	1.32 (1.01-1.71)*
	End-point	<i>Dry vs Liquid</i>	1.32 (1.01-1.72)*

^aLarger LT₅₀ written in *italics*

* Significantly different ($P < 0.05$)

Rice Weevil Pair-wise Comparisons

Formulation	Mortality Assessment	Commodity ^a	LT ₅₀ Ratio (95% CL)
Dry	Acute	Wheat vs <i>Corn</i>	1.01 (0.39-2.60)
		Wheat vs <i>Sorghum</i>	1.39 (1.04-1.87)*
		Corn vs <i>Sorghum</i>	1.38 (0.53-3.63)
	End-point	Wheat vs <i>Corn</i>	1.24 (0.44-3.49)
		Wheat vs <i>Sorghum</i>	1.64 (1.15-2.36)*
		Corn vs <i>Sorghum</i>	1.33 (0.47-3.72)
Liquid	Acute	Wheat vs <i>Corn</i>	1.19 (0.80-1.76)
		Wheat vs <i>Sorghum</i>	1.25 (0.93-1.66)
		Corn vs <i>Sorghum</i>	1.07 (0.79-1.44)
	End-point	Wheat vs <i>Corn</i>	1.57 (0.75-3.28)
		Wheat vs <i>Sorghum</i>	1.66 (0.84-3.29)
		Corn vs <i>Sorghum</i>	1.06 (0.77-1.45)

Commodity Comparisons

^a Larger LT₅₀ written in *italics*

* Significantly different ($P < 0.05$)

Conclusions

- ★ It takes significantly less time for spinosad to kill lesser grain borers than to kill rice weevils regardless of commodity, spinosad formulation, or mortality assessment
- ★ The LT_{50} s of lesser grain borers assessed immediately after exposure to spinosad are significantly higher than the LT_{50} s assessed 24 h after exposure, suggesting delayed effects
- ★ The LT_{50} s of the rice weevils were the same when mortality assessment was made immediately or 24 h later

Conclusions

- ★ There are some statistically significant differences between spinosad formulations when rice weevils were on sorghum, but the significance is small and is probably not of any practical significance
- ★ Due to the higher sorghum LT_{50} s, there were some significant differences based on commodity

Future Work

- ★ Investigate differences in lesser grain borer and rice weevil acute and end-point mortalities using radio-labeled spinosad
- ★ Examine physical differences in dry and liquid formulations on sorghum, corn, and wheat using scanning electron micrographs
- ★ Identify LT_{50} s of other stored-grain insects exposed to spinosad

Acknowledgments

- ★ Bayer (formally Gustafson) and Dow AgroSciences
- ★ Lina Metlevskiene for laboratory assistance
- ★ Insect photographs courtesy of Dr. Carl Reed
- ★ Research reported here was funded by CSREES-USDA (RAMP) under Agreement Nos. 00-51101-9674 and 2004-51001-02226

Thank you!

Questions?

Insects



Lesser grain borer (LGB)

Rhyzopertha dominica (Fabricius)



Rice weevil (RW)

Sitophilus oryzae (Linnaeus)

Methods - Grain Treatment

- ★ Liquid treated replicates: 1 mg (AI)/kg spinosad
- Dry treated replicates: 1 mg (AI)/kg spinosad
- Liquid control replicates: 1 mg/kg distilled water
- Dry control replicates: no application
- ★ 100 g of grain/replicate
- ★ 5-10 time intervals
- ★ 3 replicates, each sample treated separately
- ★ Each sample infested with 25 unsexed, mixed age adults
- ★ Incubated at 28°C and 65% RH
- ★ Completely random design