METHODOLOGY FOR RUNNING DIET-INCORPORATED BIOASSAYS WITH THE DIAMIDES

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Abstract

Diamides are a new class of insecticides recently registered in the U.S. that have excellent activity against most lepidopteran insects. Belt (flubendiamide) was the first diamide insecticide labeled for use in soybeans and has been used for 3 seasons in Mississippi. This diamide chemistry is also used in cotton production along with other row crops throughout the Mid-South. This insecticide can be characterized as having high levels of acute toxicity to most caterpillar pests. Additionally, this insecticide provides long residual control. In comparison to other labeled insecticides where residual toxicity is measured in days, the residual toxicity of the diamide class is often measured in weeks. The high levels of acute toxicity and long residual life make this class of chemistry especially vulnerable to the development of resistance by one or more species in a relatively short period of time. The focus of this project was to determine the best method for testing the susceptibility of *Helicoverpa zea* (Boddie), *Spodoptera exigua* (Hübner) and *S. frugiperda* (Smith) populations with concentration mortality bioassays. Previous work has been done in this area; however, the criterion for determining mortality was similar to that used for insecticides that act on the nervous system. In this study, larval weights were used to determine mortality.

Introduction

Bollworm, *Helicoverpa zea* (Boddie), beet armyworm, *Spodoptera exigua* (Hübner), and fall armyworm, *S. frugiperda* (Smith) are widely distributed polyphagous pests of numerous cultivated crops throughout the Mid-South. Historically these insects have been controlled with foliar applications of insecticides which has led to resistance and/or inconsistent control with most chemical classes including chlorinated hydrocarbons, organophosphates, carbamates, pyrethroids, and benzoylphenylureas (Brown et al. 1998, Temple et al. 2006; 2009, Yu 1992, and Lai and Su 2011).

A new class of insecticides exhibiting excellent lepidopteran control has recently been registered in the United States. Belt (flubendiamide), was the first diamide insecticide labeled for use in soybeans and has been used for 3 seasons in Mississippi.

Flubendiamide acts on insect ryanodine receptors. This is a new mode of action that exhibits high levels of acute toxicity and long residual control against target pests. An understanding of the mechanisms of resistance to this class of chemistry is needed to evaluate the risk of resistance development, predict levels of control that can be expected if or when resistance develops, design management plans to control resistant populations, and develop an effective resistance management plan to delay and/or prevent resistance. Since this is a new class of chemistry, little work has been done in these areas. Development of concentration-mortality responses to the diamides is necessary to provide baseline information for future resistance monitoring efforts for these insect pests (Cook et al. 2004).

Previous studies have been conducted to determine concentration-mortality relationships between lepidopteran pests and the diamide insecticides. Those studies used criteria for rating mortality similar to that used for insecticides that affect various targets in the nervous system (Hardke et al. 2011). Based on preliminary results, those criteria may not be appropriate for insecticides targeting other systems (i.e. diamide affect the muscular system). A characteristic of toxicity from a diamide such as flubendiamide is rapid feeding cessation (Nauen et al. 2007) (Photo 1-2). As a result, mortality often occurs several days after exposure due to starvation.

Photo 1-2: *S. exigua* on treated (left) and untreated (right) diet 7 DAE

The first objective of this study was to develop a concentration-mortality bioassay protocol of previously discussed Lepidopteran species that has the characteristics of low control mortality, ease of rating, repeatability , and relevance to field control. A second objective was to determine baseline susceptibility of the previously discussed Lepidoptera to diamides using this new bioassay protocol.

Materials and Methods

Bioassays were conducted on insecticide-susceptible laboratory strains and colonies derived from field populations of *H. zea*, *S. exigua* and *S. frugiperda* reared at the Delta Research and Extension Center in Stoneville, MS. These colonies were reared on a meridic semi-solid diet (Ward's Natural Science, Rochester, NY) prepared according to manufacturer's recommendations. Rearing conditions consisted of 14:10 light-dark photoperiod, 23.9-29.4˚C and 80% relative humidity (Cook et al. 2004). All bioassays on field populations were conducted within 2 generations of collection on neonate larvae (<12 h old).

Formulated Belt insecticide was dissolved into distilled water to create a stock solution of 1 µg ai/ml. Serial dilutions were then created by combining distilled water, formalin, acetic acid and the appropriate amount of stock solution (Table 1).

Table 1: Ingredients for insecticide incorporated diet concentration mortality bioassays.

Concentration	Diet	Water	Formalin	Acetic Acid	Stock Solution
0 µg ai/ml	115.2 g	363.8 ml	.36 ml	.6 ml	0 ml
0.0078 ug ai/ml	115.2 g	360.06 ml	.36 ml	.6 ml	3.74 ml
0.00975 ug ai/ml	115.2 g	359.12 ml	.36 ml	.6 ml	4.68 ml
0.01365 ug ai/ml	115.2 g	357.25 ml	.36 ml	.6 ml	6.55 ml
0.02345 ug ai/ml	115.2 g	352.57 ml	.36 ml	.6 ml	11.23 ml
0.0313 ug ai/ml	115.2 g	348.84 ml	.36 ml	.6 ml	14.96 ml
0.0469 ug ai/ml	115.2 g	341.32 ml	.36 ml	.6 ml	22.48 ml
0.0625 ug ai/ml	115.2 g	333.8 ml	.36 ml	.6 ml	30 ml
0.125 ug ai/ml	115.2 g	303.8 ml	.36 ml	.6 ml	60 ml

A methodology similar to Temple et al. (2009) was used for preparing diet-incorporated bioassays. Insecticide treated diet was then placed into 128-well bioassay trays with one larva per well and labeled according to dose and location.

Insect mortality was evaluated 7 d after exposure by recording the weight of each individual larva. Bioassay data were analyzed based on weight ranging from 1 mg – 10 mg using probit analysis (Proc Probit SAS version 9.3) to determine the best fit. Fiducial limits that did not overlap were considered significantly different. Data were corrected for control mortality using Abbot's formula (Abbot 1925).

Results and Discussion

A chi-square test was used to determine goodness of fit with a minimum acceptable fit when $p > .10$. Larvae were considered when they weighed <10 mg (*H. zea* and *S. exigua*) or< 8 mg (*S. frugiperda*). These weights were chosen because at these weights the larvae were large enough to visibly distinguish from very small larvae, yet small enough to result in low control mortality. Goodness of fit was acceptable at these weights, and is the basis for using different weights for the different species. Presented are the LC_{50}s and LC_{90}s for 2 *H. zea* colonies, 2 *S. exigua* colonies, and 3 *S. frugiperda* colonies using larval weight to determine mortality (Figure 1-3).

The LC_{50} values for *H. zea* colonies ranged from 0.016 – 0.024 ppm and LC_{90} values ranged from 0.021 – 0.033 ppm (Figure 1). The LC_{50} values for *S. exigua* colonies ranged from 0.012 – 0.017 ppm and LC_{90} values ranged from 0.017 – 0.022 ppm (Figure 2). The LC_{50} values for *S. frugiperda* colonies ranged from 0.032 – 0.042 ppm and LC_{90} values ranged from 0.045 – 0.056 ppm (Figure 3).

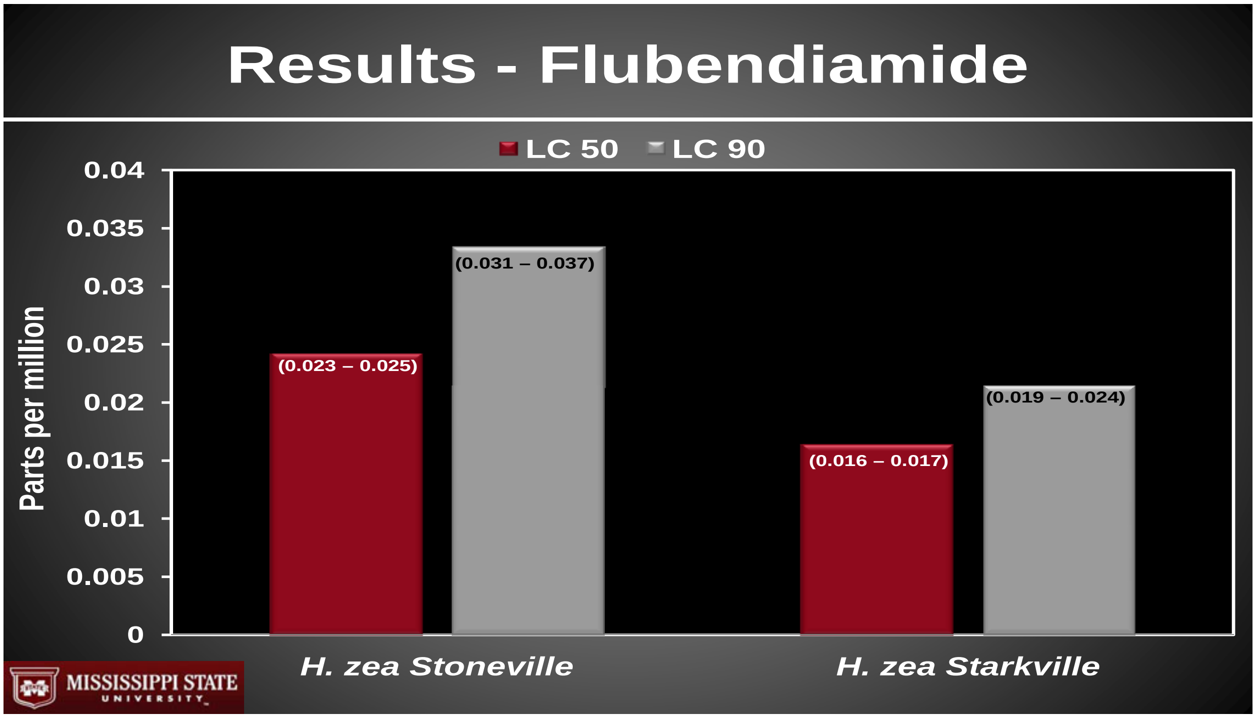

Figure 1: LC_{50} and LC_{90} of H. zea collected in Stoneville and in Starkville. Numbers within bars represent 95% fiducial limits.

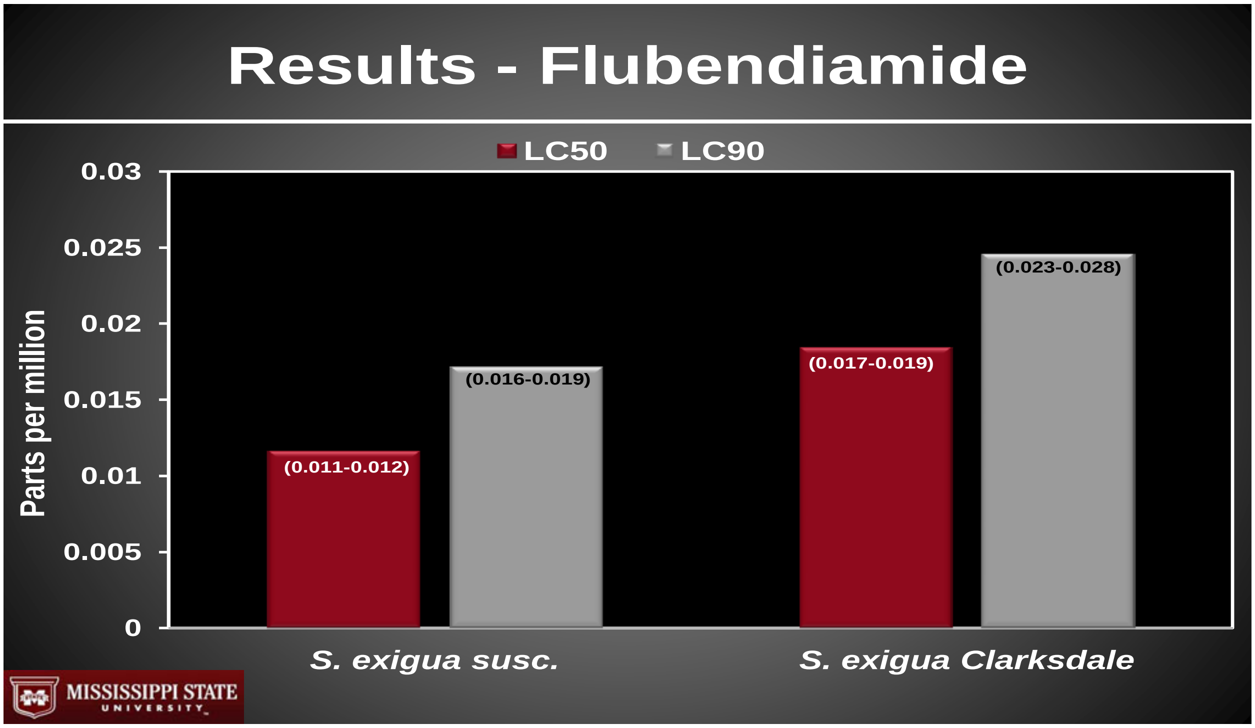

Figure 2: LC_{50} and LC_{90} of *S. exigua* from a susceptible colony and from Clarksdale, MS. Numbers within bars represent 95% fiducial limits.

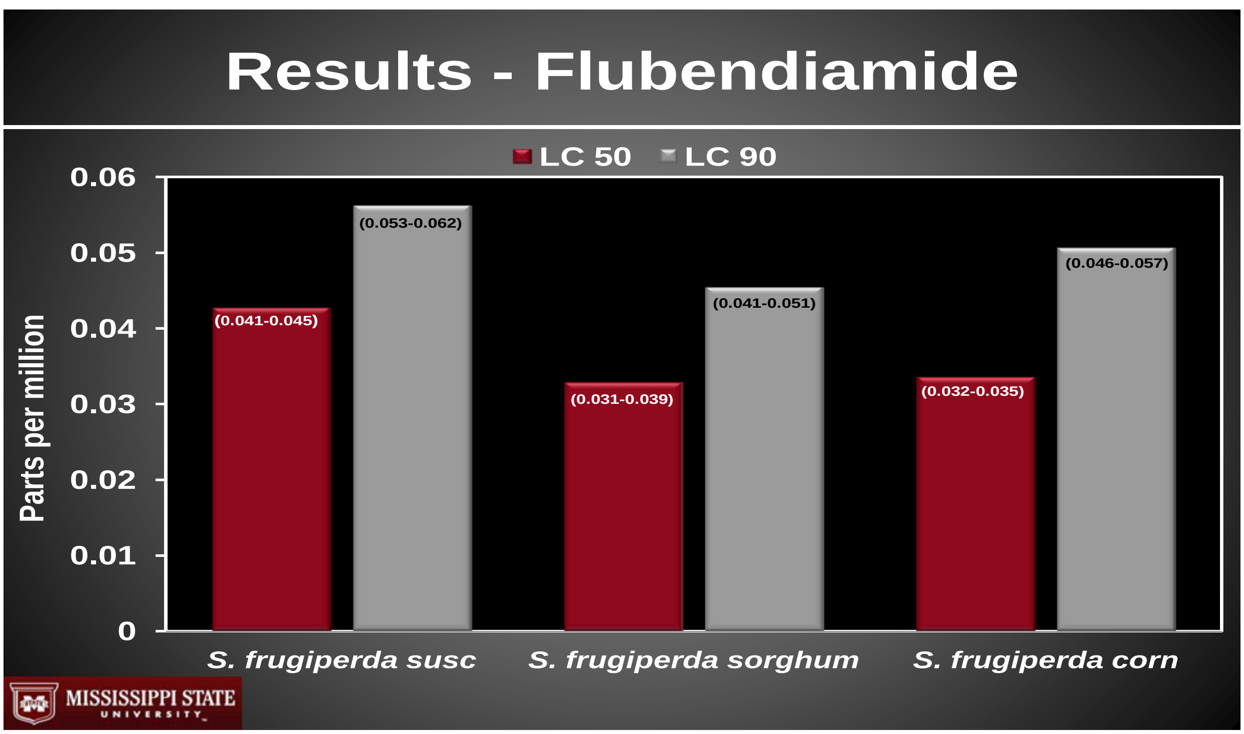

Figure 3: LC_{50} and LC_{90} of *S. frugiperda* from a susceptible lab colony, from a sorghum collection, and from corn. Numbers within bars represent 95% fiducial limits.

These data suggest that the criterion for rating mortality is appropriate for concentration-mortality bioassays with the diamide chemistry. Furthermore, because the susceptible colonies varied little from the field collected colonies, it appears that resistance to these chemistries has not yet developed among the tested populations.This information will be used as a reference for future resistance monitoring with the diamides.

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