MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 37-2015
2015 Final Report

Title: Characterization of the Resistance Potential for the Diamide Insecticides, Belt (flubendiamide) and Prevathon (chlorantraniliprole).

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SUMMARY

Insecticides in the diamide class have a novel mode of action and have become a key component in management of agriculturally important lepidopteran pests since their introduction in 2008.

Corn earworm and the armyworm complex, including fall armyworm and beet armyworm, are significant pests of agroecosystems in the Midsouthern and Southeastern regions of the United States. These insects have developed resistance to, and/or inconsistent control has occurred with most chemical classes of insecticides.

The objectives of this study were to establish baseline susceptibility levels of field populations of bollworm, fall armyworm, and beet armyworm collected in the Midsouthern and Southeastern regions of the United States to flubendiamide (Belt) and chlorantraniliprole (Prevathon).

To achieve equivalent levels of mortality for each species, a higher concentration of flubendiamide was required compared to chlorantraniliprole.

Furthermore, two experiments were conducted to determine the systemic and residual efficacy of chlorantraniliprole and flubendiamide against bollworm on vegetative and reproductive structures of soybean. Chlorantraniliprole moved systemically and provided significantly greater control than flubendiamide in the systemic and residual study out to 31 DAT. Flubendiamide did not move systemically but provided significant residual control out to 31 DAT compared with the untreated control. Neither insecticide was detected in reproductive structures.

Finally, to determine the risk of resistance development to the diamide insecticide class, a beet armyworm colony, originating from a field collection in 2013, was separated into three cohorts that were independently selected with three concentrations of flubendiamide incorporated into a meridic diet. These concentrations were chosen based on low, moderate, and high levels of mortality to the original colony. Resistance ratios never increased past 2.11-fold. The highest resistance ratios occurred after 18 generations for the low selection pressure colony, 19 generations for the moderate selection pressure colony, and 13 and 15 generations for the high selection pressure colony. After reaching their highest point of resistance, the colonies began to decline in egg production and larval survivability and did not recover. After 22 generations, the selected colonies were terminated. The results from this portion of the study suggest that the potential for resistance development of beet armyworm to flubendiamide is unclear.

REPORT OF PROGRESS

Objective 1: Quantify the variation in the response of bollworm to the diamide insecticides.

Objective 2: Determine the influence of selection pressure with the diamide class of insecticides (Belt and Prevathon) on resistance development in bollworm and beet armyworm.

Objective 3: Determine the residual and systemic activity of Belt and Prevathon in vegetative and reproductive stage soybean. (This was a contingency study because resistant populations were never identified to complete objectives 3 and 4).

Results

Objective 1. A total of 16 bollworm populations were tested to determine their susceptibility to both Belt and Prevathon. The dose required to kill 90% of each population ranged from 4.43 to 9.17 parts per trillion for Prevathon, and 21.22 to 37.49 parts per trillion for Belt. These are remarkably low levels and indicate that bollworms are highly susceptible to both of these insecticides.

In general, bollworms were more sensitive to Prevathon than to Belt. Mortality levels of all of the field populations were similar to the laboratory-susceptible population, suggesting that no resistance has been detected in any of those populations. Similar results were observed for both armyworm species tested. Both armyworm species were more tolerant to these insecticides compared to bollworms. Field populations did not show resistance to either of these insecticides.

Overall, all of these species are very susceptible to both Belt and Prevathon. Across all species and both insecticides, the doses required to kill 90% of the populations ranged from 4.43 to 48.12 part per trillion. Although this represents about a 10-fold range in variability, all species are highly susceptible to both insecticides. To put these values into perspective, 1 part per billion would be equal to 1 kernel of corn in a 50 acre field with a 250 bushel per acre yield assuming 80,000 kernels per bushel. Similarly, 1 second in 32 years would be equal to 1 part per billion.

Objective 2: This objective was initiated in 2013 with a colony of beet armyworm collected from soybean in Stoneville, MS. Colonies were selected at three different concentrations of Belt that represent low, moderate, and high doses to determine the influence of selection pressure on the evolution of resistance. A total of 22 generations were selected over the 3 years of this study. Although tolerance increased slightly at the 2 highest concentrations, it was not stable and a resistant colony was never selected.

Objective 3: Multiple experiments were conducted from 2013-2015 to determine the residual and systemic activity of Belt and Prevathon at different growth stages of soybean. This was done as a contingency plan because no resistant colonies were identified for the original objectives. In the first experiment, the residual and systemic activity of Prevathon and Belt was evaluated for an application at the R3 growth stage. Prevathon provided greater than 90% mortality on terminal leaves (newly emerged) out to 31 days after application. This suggests that Prevathon moved to tissues that were not present at the time of application. In contrast, bollworm mortality was low on newly emerged leaves, suggesting that it does not move throughout the plant. Both insecticides produced high levels of mortality on leaves that were present at the time of application. This suggests that both insecticides can provide long residual control of lepidopteran pests in soybean. However, Prevathon resulted in greater mortality than Belt at 24 and 31 days after treatment, suggesting it provides better residual control.

In a separate experiment, the systemic activity of Prevathon was tested at the V4 growth stage, and little systemic activity was observed. At 7 days after treatment, bollworm mortality was significantly higher where Prevathon was applied compared to the untreated control. However, bollworm mortality from Prevathon was less than 35%. No differences were observed at 14 days after treatment. This is probably the result of a higher rate of vegetative growth at the V4 stage compared to the R3 stage, where significant systemic activity was observed. It is assumed that the insecticide became rapidly diluted in the plant at the V4 growth stage.

In a separate test, neither Belt nor Prevathon resulted in significantly greater levels of mortality compared to the untreated control in R5.5 beans when the insecticides were applied at the R3 growth stage. This suggests that neither of these insecticides move into the reproductive structures of soybean plants.

Finally, a greenhouse experiment was conducted to better understand the systemic movement of Prevathon in soybeans. Prevathon was applied to whole plants, only the stems of plant, only the leaves of plants, and only the leaf petioles of plants. Bioassays were conducted on newly emerged leaves as a way to measure the systemic movement throughout the plant. The greatest level of mortality was observed when Prevathon was applied to the whole plant. Significant mortality was also observed when Prevathon was applied only to the stems, but not when it was applied only to the leaves and leaf petioles. This suggests that the majority of systemic activity observed with Prevathon occurs as a result of contact with the stems of soybean plants. However, mortality appeared to be additive and this suggests that adequate spray coverage is needed to maximize the systemic activity of Prevathon in soybean.

IMPACTS AND BENEFITS TO MISSISSIPPI SOYBEAN PRODUCERS

The diamide insecticides are a remarkable chemistry that provide long residual control of caterpillar pests. The residual activity of these insecticides makes them especially susceptible to resistance because multiple generations can be exposed from a single application. Because of their importance for insect control in Mississippi soybean, it is important to understand the development, mechanisms, and heritability of resistance in key insect pests.

Results from the laboratory bioassays will be important to compare future populations to, if field control failures occur, to determine if those failures are due to resistance or some other cause. Additionally, this research will help us identify factors to help detect, manage, and mitigate resistant populations in the future. Additionally, the field results will help us identify the optimum use strategies for these insecticides in Mississippi soybeans. For instance, these results suggest that when applications are needed during the early reproductive stages, Prevathon may be the best option because of the systemic activity even though it may be more expensive. In contrast, Belt may be a better option later in the reproductive stages because the systemic movement may not be needed and it can be applied at a lower cost. Overall, results from these experiments will be important for improving our recommendations to soybean growers in Mississippi.

END PRODUCTS

Graduate student Ph.D. Dissertation (Link will be provided when available)

Results from this research have been presented by the student at numerous scientific meetings, including the Mississippi Entomological Association, Entomological Society of America National and Southeastern Branch meetings, and the Beltwide Cotton Conferences.

Laboratory and field results have been presented by MSU research and extension personnel at numerous field days, workshops, and consultant and grower meetings.

One manuscript has been submitted to the Journal of Economic Entomology and a second manuscript is currently in preparation.

At least one extension publication will be published at the end of the project.

Multiple posts pertaining to this research have been and will continue to be included on the Mississippi Crop Situation Blog (www.mississippi-crops.com).

Appendix 1. Detailed results of Objective 1.

Table 1. Comparative susceptibility of bollworm, fall armyworm, and beet armyworm to Prevathon based on dose-mortality curves.

chlorantraniliprole							
Colony	**Species**	**N[1]**	**LC_{50} (95% C.L.)[2] (ng/ml)**	**LC_{90} (95% C.L.)[2] (ng/ml)**	**Slope (±SE)**	**X^2 (df)**	***P*[3]**
LAB	*H. zea*	991	3.58 (3.41-3.73)	5.25 (5.01-5.55)	3.35 (±0.24)	6.13 (5)	0.2935
AR14	*H. zea*	256	3.38 (2.85-3.76)	5.32 (4.79-6.23)	2.83 (±0.48)	3.86 (5)	0.57
GA14	*H. zea*	384	3.09 (2.81-3.32)	4.52 (4.20-5.00)	3.37 (±0.43)	6.27 (5)	0.2808
LA14	*H. zea*	288	3.84 (2.85-4.25)	5.61 (5.17-6.88)	3.38 (±0.94)	5.25 (3)	0.1541
MSKIL14	*H. zea*	634	4.21 (4.02-4.39)	5.86 (5.56-6.28)	3.82 (±0.37)	8.57 (5)	0.1275
MSLEL14	*H. zea*	448	3.79 (3.44-4.14)	7.61 (6.57-9.54)	1.84 (±0.23)	5.73 (4)	0.2202
MSSTARK13	*H. zea*	384	4.11 (3.50-4.77)	9.17 (7.53-12.32)	1.60 (±0.21)	3.89 (5)	0.5659
MSSTARK14	*H. zea*	512	3.62 (3.42-3.84)	4.98 (4.54-5.86)	4.02 (±0.64)	1.56 (5)	0.9059
MSSTONE13-1	*H. zea*	881	2.94 (2.75-3.11)	4.43 (4.07-5.03)	3.13 (±0.40)	4.93 (4)	0.2947
MSSTONE13-2	*H. zea*	1643	3.21 (3.05-3.36)	5.10 (4.74-5.61)	2.76 (±0.23)	4.44 (5)	0.4882
MSSTONE13-3	*H. zea*	1166	3.52 (3.36-3.68)	4.68 (4.35-5.27)	4.51 (±0.64)	6.09 (5)	0.2974
MSYAZ 14	*H. zea*	128	4.09 (3.25-4.84)	7.47 (6.15-10.72)	2.12 (±0.43)	8.43 (5)	0.134
NC14	*H. zea*	256	4.22 (3.86-4.49)	5.32 (4.98-5.91)	5.54 (±1.01)	6.38 (5)	0.2702
SC14	*H. zea*	384	4.05 (3.76-4.26)	5.06 (4.8-5.49)	5.72 (±0.93)	9.07 (5)	0.1064
TN14-1	*H. zea*	256	3.72 (3.37-3.98)	4.76 (4.44-5.32)	5.21 (±0.96)	0.43 (5)	0.9946
TN 14-2	*H. zea*	256	3.15 (2.19-3.67)	5.26 (4.65-6.53)	2.49 (±0.61)	6.83 (5)	0.2336
LAB	*S. frugiperda*	780	6.78 (6.46-7.08)	10.27 (9.72-11.00)	3.09 (±0.23)	6.63 (4)	0.1566
MSSTARK13	*S. frugiperda*	1112	6.16 (5.85-6.44)	8.95 (8.46-9.60)	3.43 (±0.28)	4.86 (5)	0.4334
MSSTONE13	*S. frugiperda*	377	6.19 (5.79-6.60)	9.06 (8.32-10.19)	3.37 (±0.36)	5.35 (5)	0.3741
LAB	*S. exigua*	913	6.71 (6.30-7.11)	10.59 (9.85-11.60)	2.81 (±0.23)	1.91 (3)	0.5907
MSCLARK13	*S. exigua*	331	13.30 (11.48-14.63)	20.70 (18.49-25.52)	2.89 (±0.56)	0.69 (2)	0.7066

Table 2. Comparative susceptibility of bollworm, fall armyworm, and beet armyworm to Belt based on dose-mortality curves.

			flubendiamide				
Colony	**Species**	**N[1]**	**LC_{50} (95% CL)[2] (ng/ml)**	**LC_{90} (95% CL)[2] (ng/ml)**	**Slope (±SE)**	**X^2 (df)**	***P*[3]**
LAB	*H. zea*	447	21.96 (20.36-23.21)	29.12 (27.71-30.95)	4.54 (±0.53)	5.39 (4)	0.2497
AR14	*H. zea*	256	21.88 (19.23-56.58)	29.59 (27.58-33.19)	4.25 (±0.81)	6.29 (5)	0.2789
GA14	*H. zea*	256	27.75 (26.23-28.99)	34.79 (32.67-39.24)	5.66 (±1.06)	3.35 (5)	0.6459
LA14	*H. zea*	256	23.34 (21.85-24.66)	30.47 (28.65-33.15)	4.80 (±0.60)	7.27 (5)	0.2010
MSKIL14	*H. zea*	398	29.34 (27.58-30.48)	35.33 (34.07-37.38)	6.89 (±1.13)	0.70 (2)	0.7042
MSLEL14	*H. zea*	384	24.43 (21.82-25.90)	32.87 (30.86-37.31)	4.32 (±0.90)	9.06 (5)	0.1068
MSNAT 14	*H. zea*	192	24.04 (19.94-26.28)	30.76 (28.57-33.74)	5.22 (±1.16)	2.68 (3)	0.4439
MSSTARK13-1	*H. zea*	406	17.02 (16.19-17.91)	21.75 (20.37-23.88)	5.23 (±0.61)	1.25 (3)	0.5354
MSSTARK14-1	*H. zea*	256	16.45 (15.25-17.77)	21.22 (19.29-25.73)	5.04 (±1.03)	1.56 (5)	0.906
MSSTONE13-1	*H. zea*	947	19.72 (18.54-20.83)	32.38 (30.28-35.21)	2.58 (±0.20)	3.28 (3)	0.1938
MSSTONE13-2	*H. zea*	672	21.23 (15.30-23.73)	33.82 (30.75-43.90)	2.75 (±0.76)	4.60 (3)	0.2035
MSVIC14	*H. zea*	320	25.19 (22.51-26.86)	29.55 (27.91-31.13)	8.04 (±1.43)	1.62 (2)	0.4443
MSYAZ14	*H. zea*	560	30.74 (29.99-31.52)	37.49 (35.95-39.87)	6.46 (±0.72)	1.03 (3)	0.7948
NC14	*H. zea*	288	24.47 (23.22-25.36)	28.19 (27.23-29.60)	9.07 (±1.46)	2.60 (3)	0.4572
SC14	*H. zea*	896	25.70 (25.14-26.22)	30.58 (29.79-31.61)	7.37 (±0.60)	9.23 (5)	0.1002
TN14-1	*H. zea*	256	22.82 (20.37-24.64)	32.20 (29.66-36.70)	3.72 (±0.63)	3.85 (5)	0.5718
TN14-2	*H. zea*	256	22.30 (20.24-23.80)	28.55 (26.74-31.49)	5.19 (±0.90)	4.34 (5)	0.5009
LAB	*S. frugiperda*	780	33.63 (31.99-35.22)	48.12 (45.39-51.77)	3.57 (±0.29)	1.03 (3)	0.7938
MSSTARK13	*S. frugiperda*	975	30.73 (29.60-31.87)	43.62 (41.39-46.53)	3.66 (±0.25)	4.04 (3)	0.2568
MSSTONE13	*S. frugiperda*	1428	34.01 (31.52-34.11)	46.84 (44.78-49.40)	3.61 (±0.23)	1.39 (3)	0.7058
LAB	*S. exigua*	1197	12.40 (11.64-13.15)	20.31 (18.86-22.24)	2.60 (±0.23)	4.47 (5)	0.4838
MSCLARK13	*S. exigua*	402	18.46 (17.10-19.88)	25.75 (23.59-28.94)	3.85 (±0.42)	6.80 (5)	0.2360

Appendix 2. Detailed results from Objective 3.

Table 1. Interaction between insecticide, leaf position, and days after treatment (DAT) on percent mortality of bollworms on soybean leaves sprayed at the R3 growth stage.

		Percent Mortality			
Treatment	**Leaf**	**10 DAT**	**17 DAT**	**24 DAT**	**31 DAT**
Prevathon	Upper	69a	89ab	93a	92a
Belt	Upper	15de	16de	12de	13de
Untreated control	Upper	7e	11de	7e	6e
Prevathon	Lower	98a	95a	98a	95a
Belt	Lower	97a	90ab	80b	83c
Untreated control	Lower	10de	10de	8de	9de

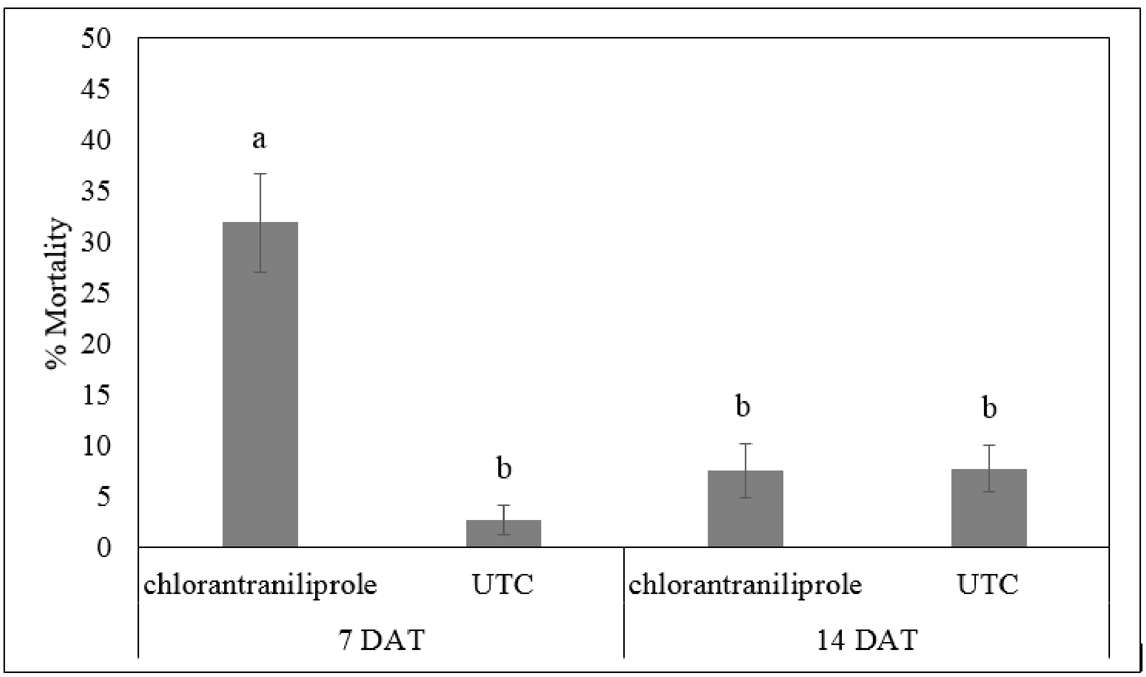

Figure 1. Mortality of bollworm exposed to leaves that developed after application of Prevathon at the V4 growth stage.

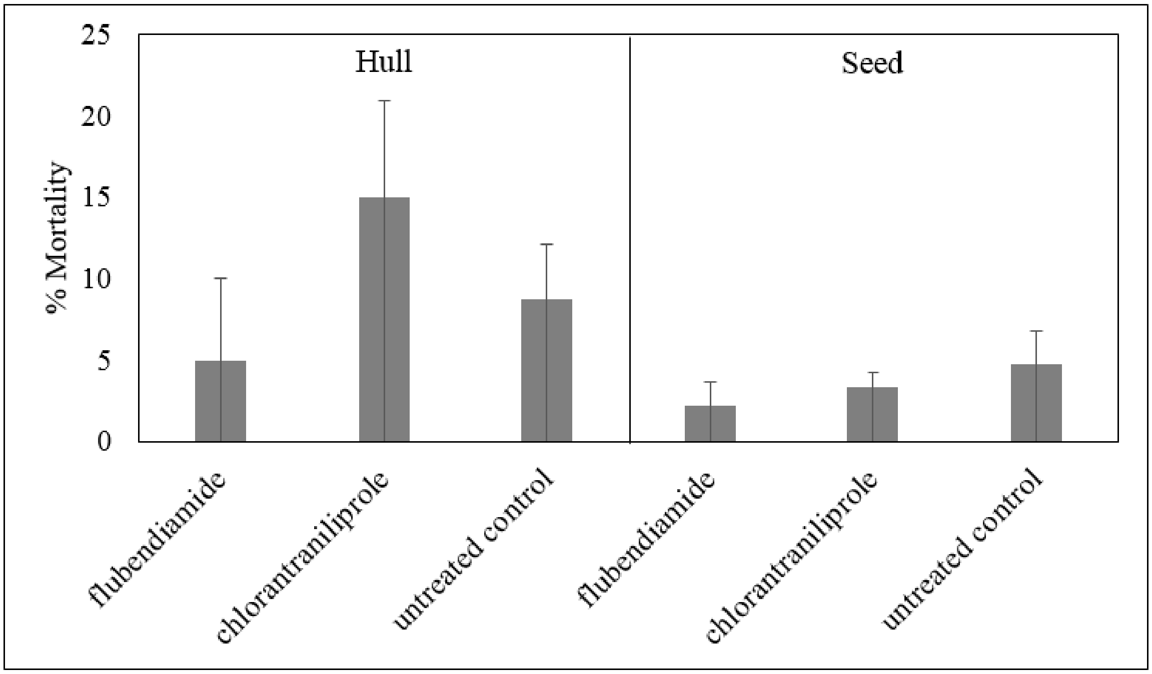

Figure 2. Percent mortality of bollworm larvae on reproductive structures of soybean at the R5.5 growth stage following application of the insecticides at the R3 growth stage.

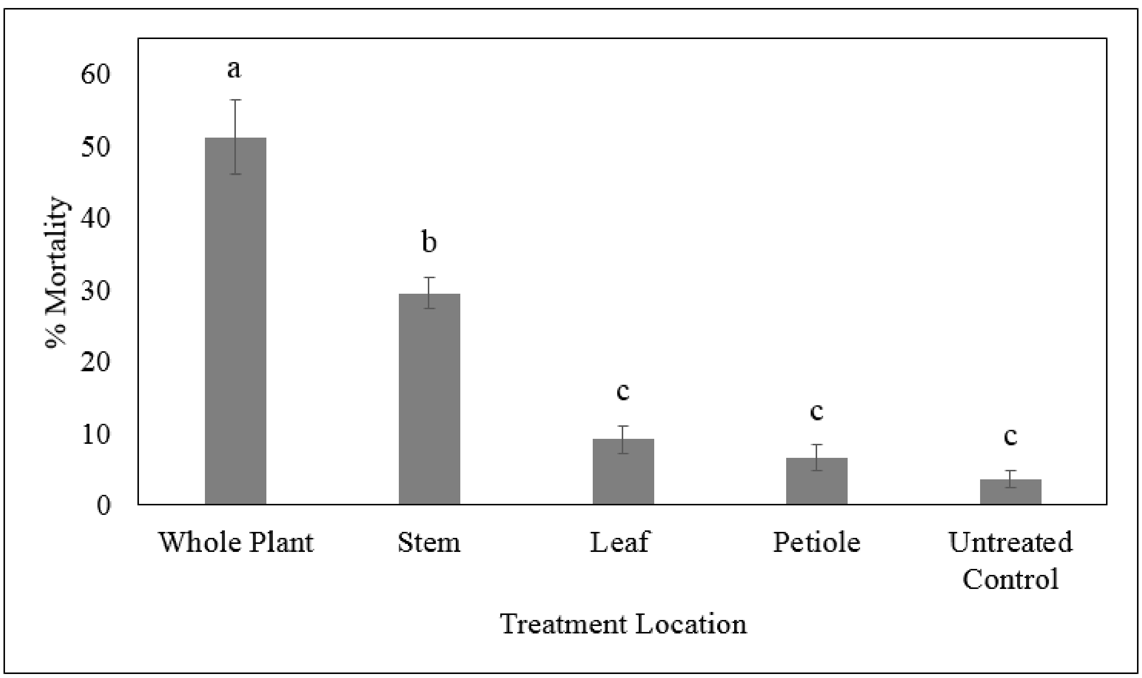

Figure 3. Percent mortality of bollworm larvae on terminal leaves of soybean where Prevathon was applied to different plant structures in the greenhouse.