

## LABORATORY EVALUATION OF SELECTED INSECTICIDES ON FIELD-COLLECTED POPULATIONS OF BOLLWORM AND TOBACCO BUDWORM LARVAE

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### Abstract

Bollworm (CEW) and Tobacco Budworm (TBW) larvae and adults were collected from a variety of host crops and evaluated for susceptibility to MVP II<sup>®</sup>, Cypermethrin and Spinosad (Tracer<sup>®</sup>) during the 2011 season. Results were compared to historical data collected throughout a seventeen-year study period beginning in 1995. As expected, CEW larvae were less susceptible to the effect(s) of MVP II<sup>®</sup> than TBW larvae. Throughout the study period, the susceptibilities of CEW and TBW larvae to MVP II<sup>®</sup> have decreased until this season. The mean 2010 MVP II<sup>®</sup> LC<sub>50</sub> value for CEW was ca. 10-fold higher than 2009 values and the mean 2010 MVP II<sup>®</sup> LC<sub>50</sub> value for TBW was ca. 4.5-fold higher than values recorded during the 2009 season; however, the mean 2011 MVP II<sup>®</sup> LC<sub>50</sub> value for CEW was ca. 49-fold lower than 2010 values and the mean 2011 MVP II<sup>®</sup> LC<sub>50</sub> value for TBW was ca. 10-fold lower than values recorded during the 2010 season. Although cypermethrin remains an effective insecticide to control CEW larvae, average LC<sub>50</sub> values were highest during the 2011 season; the average LC<sub>50</sub> value for cypermethrin against CEW larvae collected during the 2010 season was ca. 19-fold higher than the average LC<sub>50</sub> values obtained during the mid-1990s. The effectiveness of cypermethrin for the control of TBW larvae has declined throughout the seventeen-year study period also; the average LC<sub>50</sub> value for cypermethrin against TBW larvae collected during the 2011 season was ca. 61-fold higher than the LC<sub>50</sub> value obtained for a pyrethroid-susceptible laboratory strain (HRV) and ca. 20-fold higher than the average LC<sub>50</sub> values obtained during the mid-1990s. Decreases in the susceptibilities of CEW and TBW populations have been confirmed by the use of topical application bioassays (not shown) and adult vial tests. Spinosad (Tracer<sup>®</sup>) has remained highly effective against CEW and TBW larvae throughout the study period.

### Introduction

The bollworm (CEW; *Helicoverpa zea*) and the tobacco budworm (TBW; *Heliothis virescens*) are two of the more economically important pests of cotton in the United States. Because CEW and TBW populations have developed resistance to many of the insecticides used for their control, it is essential that research efforts and agricultural practices be devoted to the preservation of those insecticides that are still effective and to the development of new replacement compounds and technologies. Programs to monitor insecticide susceptibilities in field-collected populations of CEW and TBW are critical to the development of those effective management strategies. Samples of CEW and TBW populations were collected from cotton, tobacco, peanut and corn fields throughout Georgia during the summer of 2011. Larvae from those field-collected samples were assayed for susceptibility to a variety of insecticides using treated-diet and topical application bioassays. Adults were evaluated using an adult vial test bioassay. Results were compared to baseline data collected between 1995-1999, 2003-2005, and 2009-2010.

### Materials and Methods

The counties from which bollworm (CEW) and tobacco budworm (TBW) have been collected throughout the study period are shown in Figure 1. During the 2011 season, 5 CEW and 5 TBW populations were collected from 6 counties including Carroll, Clay, Decatur, Miller, Mitchell, and Tift. Field-collected CEW and TBW moths or larvae were transported to facilities at the University of West Georgia. Larvae were transferred to a pinto bean/wheat germ, agar-based diet, and adults were placed in mating cages to produce adequate numbers of larvae for testing. Larvae and adults were maintained at 27°C, LD 14:10 and ca. 40% RH. The insecticides used were MVP II<sup>®</sup> (19.1% A.I., Monsanto Corporation, St. Louis, MO); cypermethrin (94.3% A.I., FMC Corporation, Princeton, NJ); and spinosad (91.3% A.I., Dow AgroSciences, Indianapolis, IN).

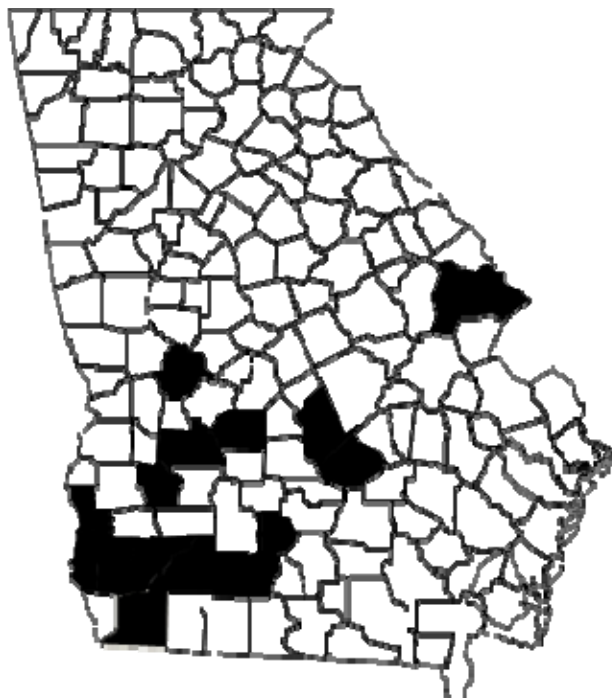


Figure 1. Bollworm and tobacco budworm collection sites.

Larvae were evaluated using a modified insecticide-treated diet bioassay or by topical application; adults were evaluated using an adult vial test (AVT) protocol. For the insecticide-treated diet assay, an insecticide test solution (100  $\mu$ l) was added to 50 mL of liquefied pinto bean/wheat germ, agar-based diet at ca. 57°C while mixing with a variable speed stirrer. The insecticide-treated diet (ca. 2.5 mL) was distributed into 1 oz. clear plastic medicine cups. The treated diets were allowed to cool and gel. One neonate or one late 2<sup>nd</sup> instar larva (depending upon the insecticide being evaluated) was added to each cup, and mortality was monitored over a 4 day period. For the topical application bioassay, a 1  $\mu$ l droplet of insecticide solution or acetone (control) was applied to the dorsal thorax of a 4<sup>th</sup> instar larva (ca. 35 mg). Mortality was assessed after a 48 h exposure period. For the adult vial test, a single moth was placed in an insecticide-treated or acetone-treated (control) vial. Mortality was assessed after a 24 h exposure period. Mortality was defined as the inability of the larva to move across the diet surface when probed or for a moth to fly a distance of 1 meter when dropped from a 2 meter height. During the treatment period, the larvae and adults were held in an environmental chamber at 27°C, LD 14:10 and ca. 40% RH.

### **Results and Discussion**

As expected, MVP II<sup>®</sup> was less effective against CEW larvae as compared to TBW larvae (Tables 1-4; Figures 1, 2, 6 & 7). The average CEW LC<sub>50</sub> value was ca. 8-fold greater than the average TBW LC<sub>50</sub> value. Isolated CEW and TBW populations have exhibited high levels of survival following exposure to MVP II<sup>®</sup>; average CEW and TBW LC<sub>50</sub> values for the 2010 season were ca. 4-fold greater than average CEW and TBW LC<sub>50</sub> values obtained during the 2009 season. However, a substantial increase in susceptibility was noted during the 2011 season; average CEW and TBW LC<sub>50</sub> values for the 2011 season were ca. 48-fold and 9-fold less than average 2010 CEW and TBW LC<sub>50</sub> values, respectively. Variability may be associated with the crop from which the populations were collected and the time during the season that the populations were collected.

Table 1. Susceptibilities of field-collected bollworm populations to MVP II<sup>®</sup>, spinosad (Tracer<sup>®</sup>), and cypermethrin using treated diet, topical application, and adult vial test (AVT) bioassays--2011

Colony	Treatment	<u>Diet</u>	<u>AVT*</u>
		LC <sub>50</sub> (C.I.; Slope), ppm	LC <sub>50</sub> (C.I.; Slope), µg/vial
CLA 11	MVPII <sup>®</sup>	10.6 (6.70-16.9; 1.54)	
DEC 11		31.7 (14.4-82.1; 1.2)	
MIL 11		2.82 (1.72-4.81; 2.08)	
MIT 11		3.44 (2.53-4.66; 1.07)	
TIF 11		2.63 (1.20-5.39; 1.02)	
MIL 11	Spinosad	1.15 (0.63-2.17; 2.26)	
TIF 11		1.52 (1.18-1.98; 2.45)	
CLA 11	Cypermethrin	13.6 (7.10-11.8; 2.26)	-----
DEC 11		51.0 (28.1-121; 1.15)	-----
MIL 11		-----	5.49 (4.70-6.45; 5.55)
TIF 11		15.7 (12.7-19.3; 3.66)	2.84 (1.82-3.96; 2.46)

\*Adult vial test (males and female adults evaluated)

Table 2. Susceptibilities of field-collected tobacco budworm populations to MVP II<sup>®</sup>, spinosad (Tracer<sup>®</sup>), and cypermethrin using treated diet, topical application, and adult vial test (AVT) bioassays—2011.

Colony	Treatment	<u>Diet</u>	<u>AVT*</u>
		LC <sub>50</sub> (C.I.; Slope), ppm	LC <sub>50</sub> (C.I.; Slope), µg/vial
DEC 11	MVPII <sup>®</sup>	0.24 (0.11-0.40; 1.86)	
MIL 11		0.40 (0.29-0.53; 1.34)	
TIF 11		1.43 (0.84-2.34; 1.19)	
TIFt 11		0.67 (0.37-1.11; 0.87)	
UWG 11		3.83 (2.51-5.73; 1.14)	
DEC 11	Spinosad	0.89 (0.57-1.42; 1.59)	
MIL 11		0.43 (0.26-0.72; 1.42)	
TIFt 11		0.34 (0.26-0.43; 1.49)	
UWG 11		0.57 (0.40-0.81; 1.29)	
DEC 11	Cypermethrin	174 (117-338; 1.46)	-----
MIL 11		54.8 (43.5-71.5; 1.82)	3.46 (2.22-5.48; 2.65)
TIF 11		89.9 (58.7-258; 1.78)	4.25 (2.71-7.03; 2.14)
TIFt 11		58.2 (44.7-83.2; 2.05)	5.10 (3.92-6.64; 1.93)
UWG 11		59.1 (52.0-68.4; 2.23)	4.06 (3.06-5.31; 2.80)

\* Adult Vial Test (males and female adults evaluated)

Table 3. Mean susceptibilities of bollworm larvae to MVP<sup>®</sup>, cypermethrin and spinosad (Tracer<sup>®</sup>) following a 96 h exposure period using an insecticide-treated diet bioassay--1996-2010.

Year	LC <sub>50</sub> , ppm (Slope)		
	MVP <sup>®</sup>	Cypermethrin	Spinosad
1996	38.9 (1.7)	1.40 (2.1)	0.30 (1.6)
1997	68.3 (1.6)	1.31 (2.2)	ND
2003	110 (0.6)*	4.49 (1.8)	0.51 (1.5)
2004	128 (1.1)*	2.63 (3.4)	0.30 (2.1)
2005	122 (0.3)*	1.13 (0.8)	ND
2009	40.1 (0.9)*	8.72 (3.3)	0.54 (3.4)
2010	494 (1.0)*	11.8 (2.6)	0.56 (2.3)
2011	10.2 (1.3)*	26.8 (3.6)	1.34 (4.0)

ND = Not Determined

\* Data based on tests using neonate larvae

Table 4. Mean susceptibilities of tobacco budworm larvae to MVP<sup>®</sup>, cypermethrin and spinosad (Tracer<sup>®</sup>) following a 96 h exposure period using an insecticide-treated diet bioassay--1995-2010.

Strain	LC <sub>50</sub> , ppm (Slope)		
	MVP <sup>®</sup>	Cypermethrin	Spinosad
HRV	ND	1.42 (5.2)	0.38 (1.4)
OPS	0.75 (0.7)	5.01 (3.2)	0.14 (3.3)
OPR	ND	5.48 (2.7)	0.37 (2.2)
PYR	1.23 (1.9)	36.5 (2.1)	0.40 (3.4)
1995	0.95 (1.0)	0.46 (1.1)	0.84 (1.7)
1996	9.63 (1.0)	4.32 (3.0)	0.48 (3.1)
1997	8.68 (1.2)	7.55 (2.5)	0.35 (1.8)
1998	ND	12.1 (1.7)	ND
1999	ND	11.5 (0.9)	0.20 (1.9)
2003	1.00 (0.5)*	33.1 (1.4)	0.52 (1.1)
2004	1.20 (1.6)*	33.1 (1.3)	0.40 (1.6)
2005	3.33 (0.5)*	27.6 (1.2)	0.32 (1.2)
2009	2.66 (1.2)*	52.7 (2.2)	0.61 (1.8)
2010	12.2 (1.3)*	69.4 (2.5)	0.43 (2.4)
2011	1.31 (1.3)*	87.2 (1.9)	0.56 (1.5)

ND = Not Determined

\* Data based on tests using neonate larvae

Decreases in the susceptibilities of CEW and TBW populations to pyrethroid insecticides have been noted throughout the study period. 2011 LC<sub>50</sub> values (for both CEW and TBW) have been the highest LC<sub>50</sub>s recorded to date (Tables 1-4; Figures 3 & 8) and averaged ca. 2-fold higher than the 2010 values, more than 9-fold greater than LC<sub>50</sub> values for CEW field populations collected during the 2003-2005 seasons, and more than 19-fold greater than LC<sub>50</sub> values obtained for field populations collected during the 1996 and 1997 seasons (Table 2; Figures 1 & 3). The average 2011 TBW LC<sub>50</sub> value was ca. 1.4-fold higher than the average 2009-2010 TBW LC<sub>50</sub> value, ca. 3-fold higher than the average 2003-2005 TBW LC<sub>50</sub> value, and 12-fold greater than the average 1995-1999 value. In addition, the average 2011 TBW LC<sub>50</sub> value was more than 61-fold higher than the LC<sub>50</sub> value obtained for the pyrethroid-susceptible HRV laboratory strain and 2.3-fold higher than the LC<sub>50</sub> value obtained for a laboratory-selected, pyrethroid-resistant strain (PYR) (Table 4). As indicated by the previously presented treated diet data, AVT data (Tables 1 & 2; Figure 5) indicated a decrease in the susceptibility of CEW and TBW populations to

cypermethrin over time also. In field-collected TBW populations, an evaluation of  $LC_{95}$  values for cypermethrin against TBW larvae indicated an annual and sharp increase since the monitoring project began (Figure 10).

To date, spinosad (Tracer<sup>®</sup>) has remained effective against all strains tested (Tables 1-4; Figures 2, 4, 6, 9 & 10). Mean  $LC_{50}$  values for bollworm larvae (0.98 ppm) and TBW larvae (0.43 ppm) were comparable and have remained relatively stable throughout the seventeen-year study period.

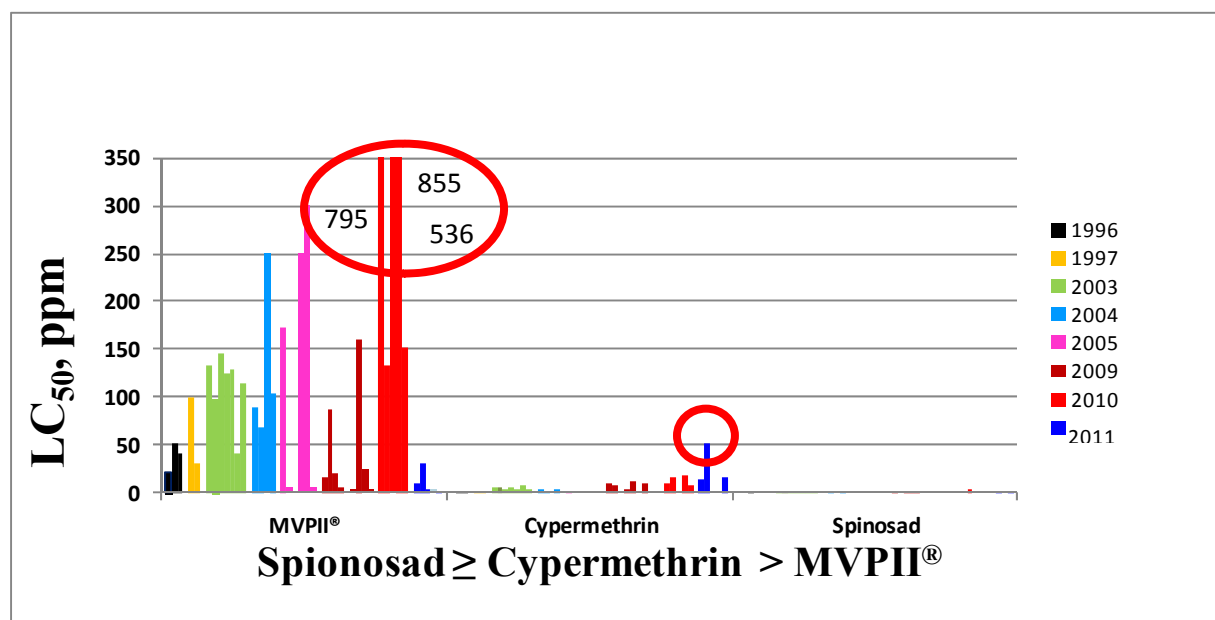


Figure 2. Susceptibilities of field-collected bollworm larvae to MVP II<sup>®</sup>, spinosad (Tracer<sup>®</sup>), and cypermethrin using a treated diet bioassay—1996-2011.

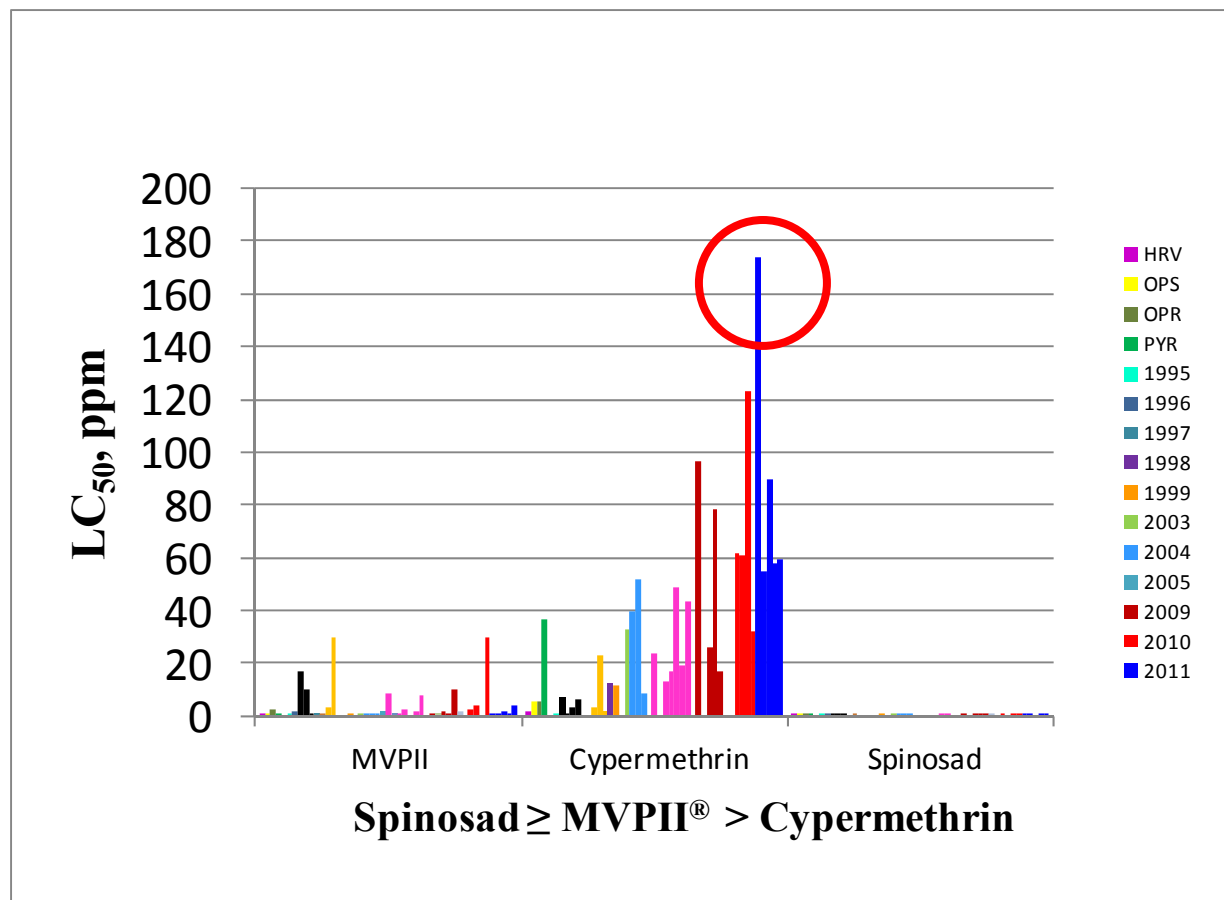


Figure 3. Susceptibilities of field-collected tobacco budworm larvae to MVP II®, spinosad (Tracer®), and cypermethrin using a treated diet bioassay—1996-2011.

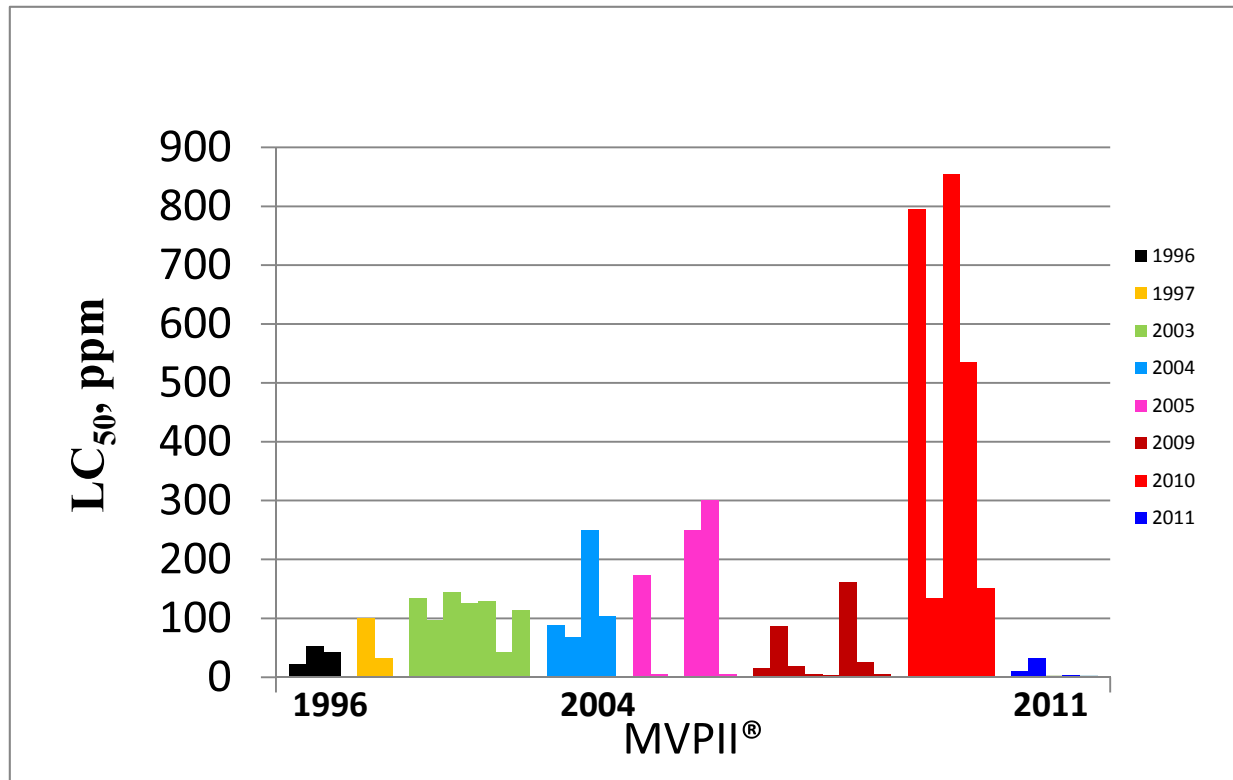


Figure 4. Susceptibilities of field-collected bollworm larvae to MVP II® using a treated diet bioassay—1996-2011.

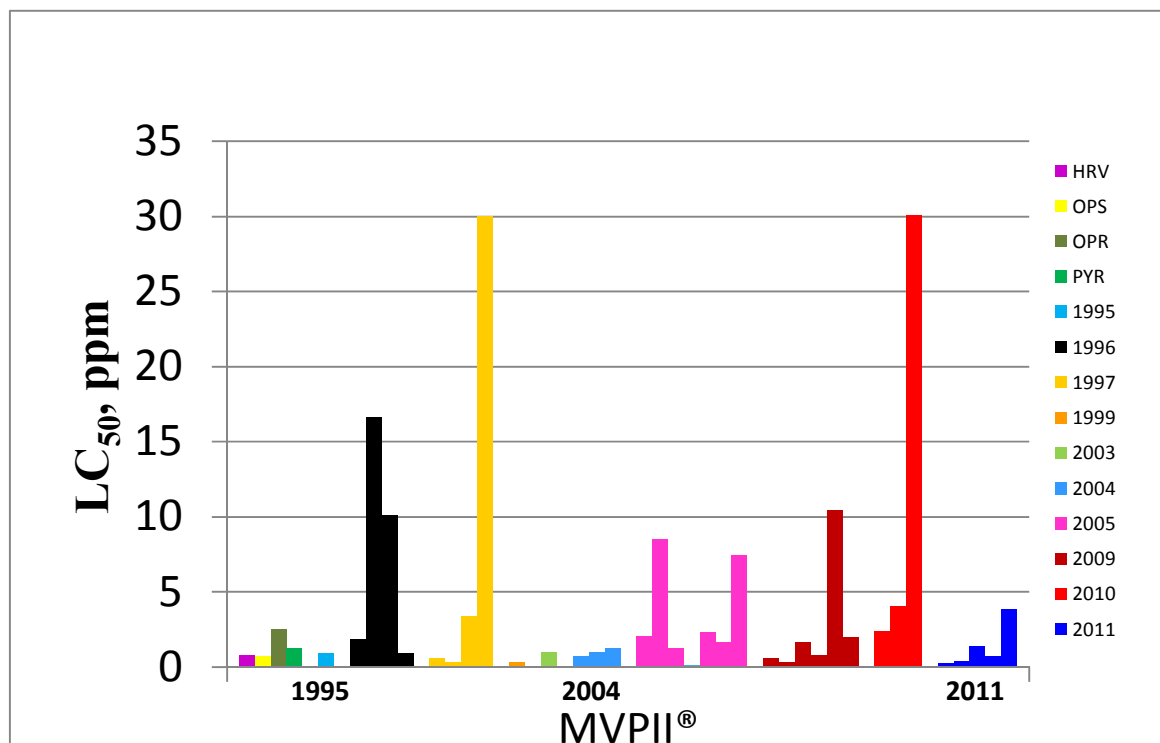


Figure 5. Susceptibilities of field-collected tobacco budworm larvae to MVP II® using a treated diet bioassay—1995-2011.

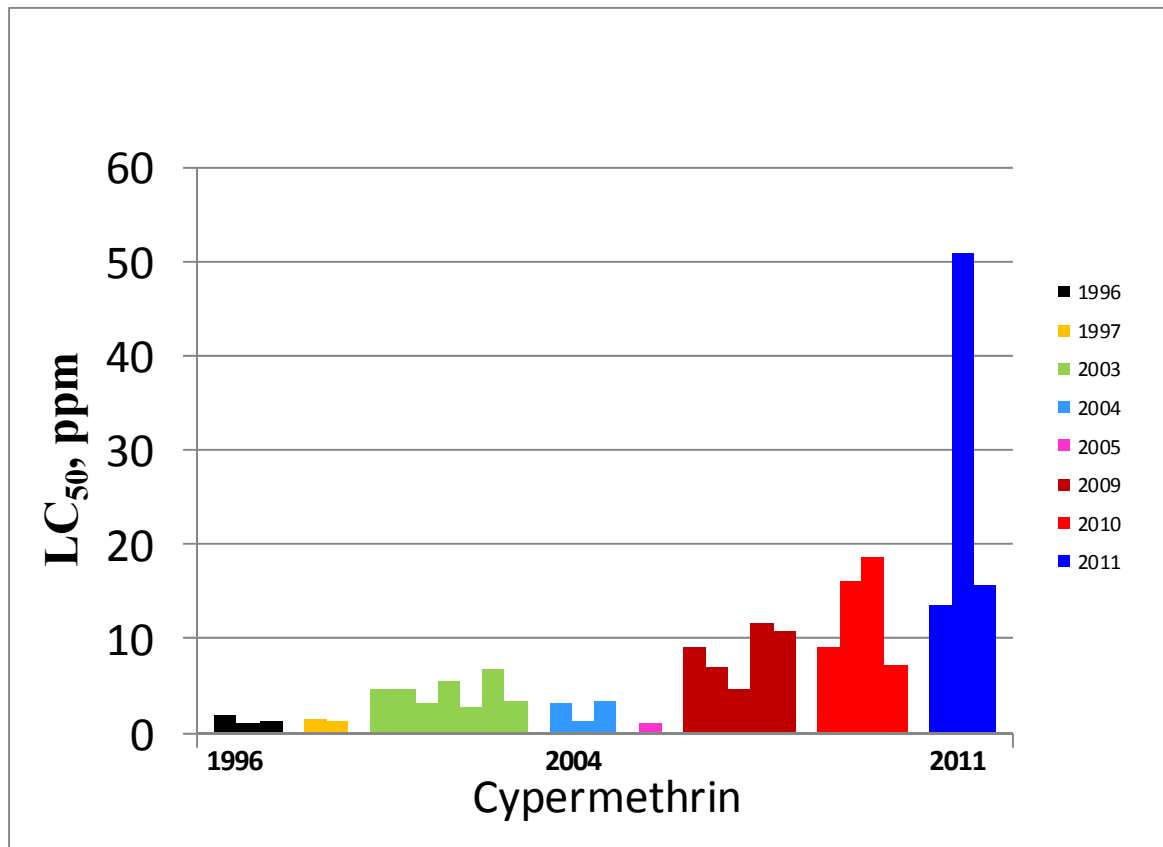


Figure 6. Susceptibilities of field-collected bollworm larvae to cypermethrin using a treated diet bioassay—1996-2011.

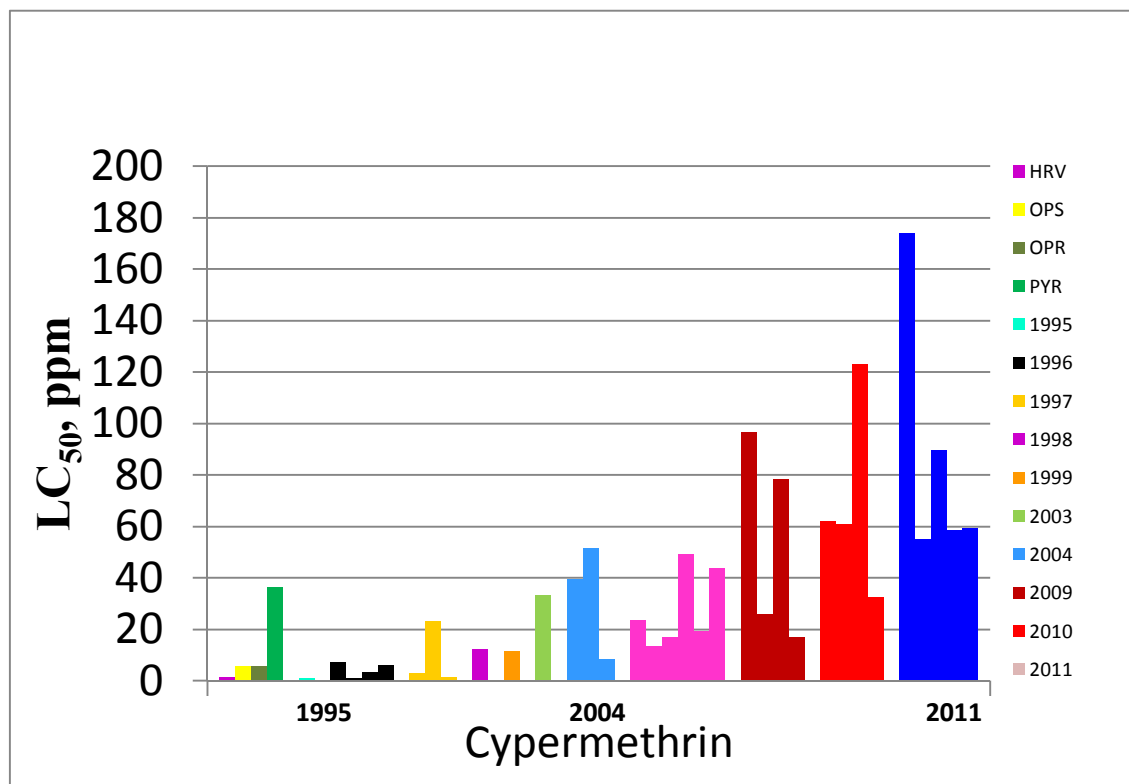


Figure 7. Susceptibilities of field-collected tobacco budworm larvae to cypermethrin using a treated diet bioassay—1995-2011.

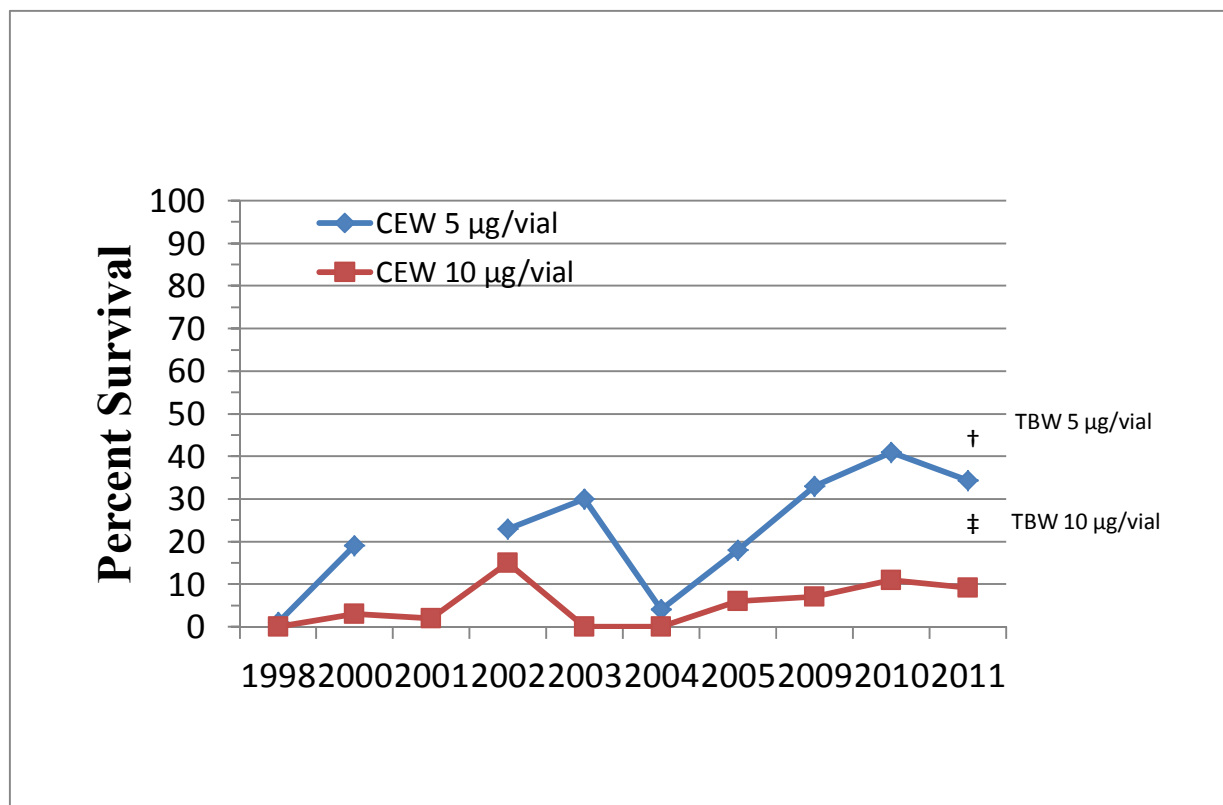


Figure 8. Susceptibilities of bollworm and tobacco budworm adults to cypermethrin using an adult vial test (AVT) bioassay—1998-2011.

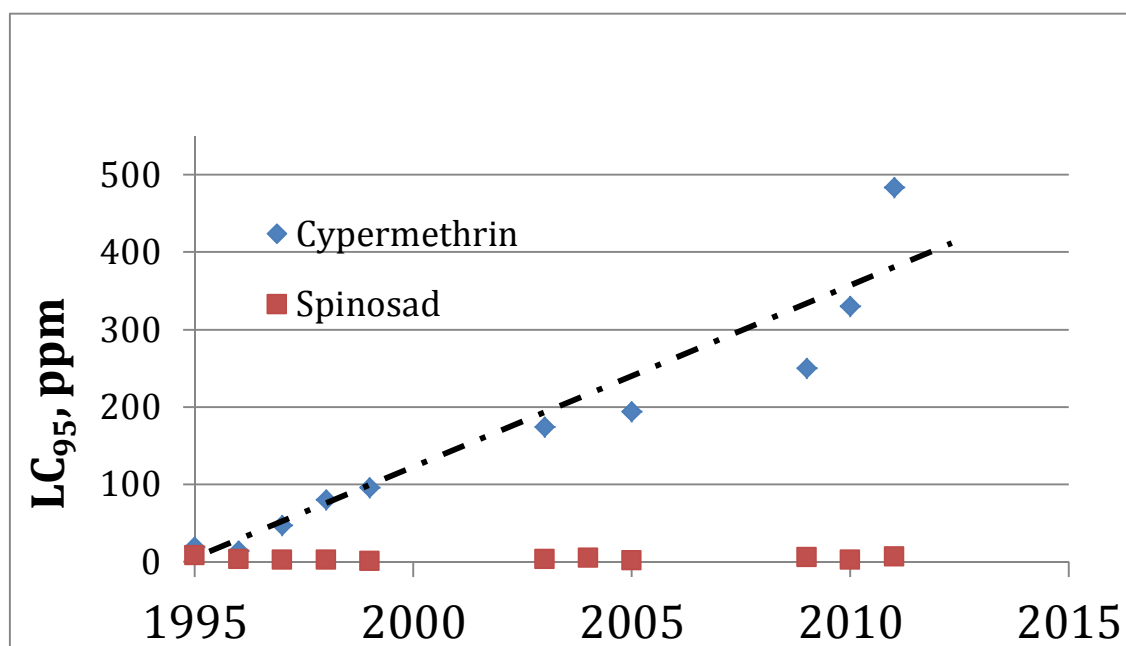


Figure 9. Susceptibilities of bollworm larvae to cypermethrin and spinosad (Tracer®) expressed as the LC<sub>95</sub> using a treated diet bioassay—1995-2011.

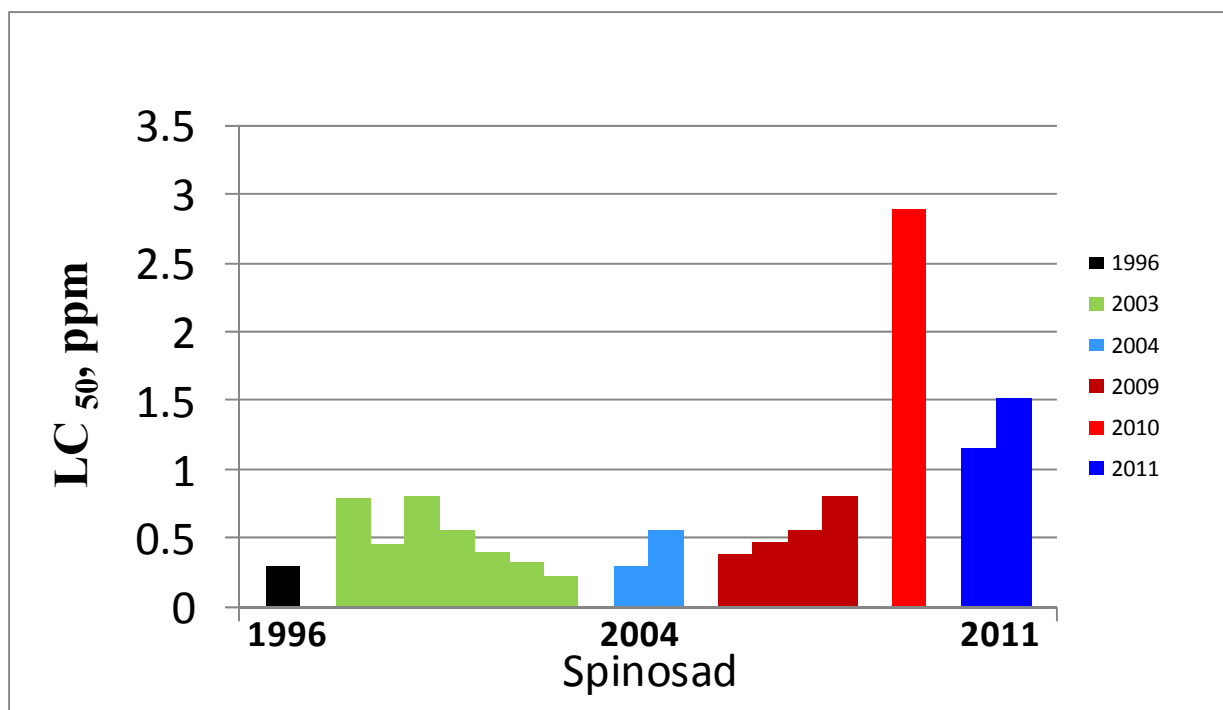


Figure 10. Susceptibilities of field-collected bollworm larvae to spinosad (Tracer®) using a treated diet bioassay—1996-2011.

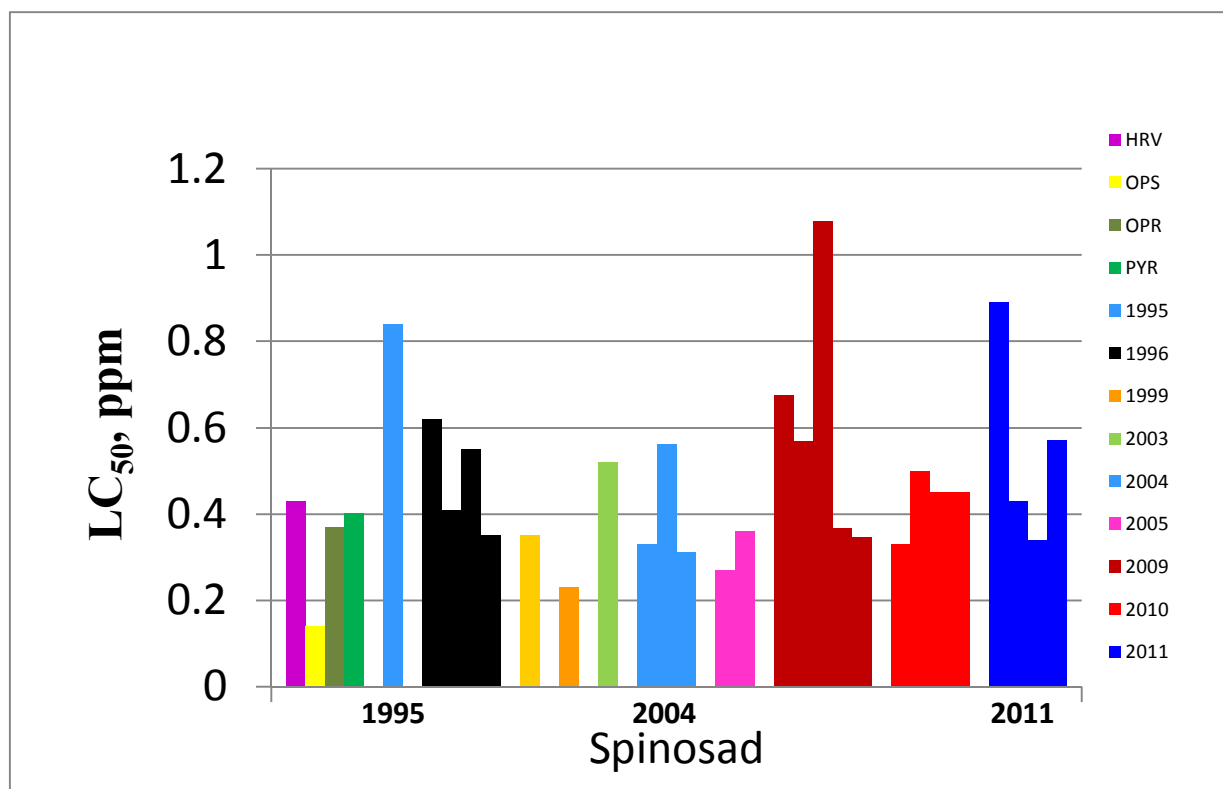


Figure 11. Susceptibilities of field-collected tobacco budworm larvae to spinosad (Tracer®) using a treated diet bioassay—1996-2011.

### **Summary**

Throughout the seventeen year study period bollworm (CEW) and tobacco budworm (TBW) populations in Georgia have remained relatively susceptible to MVP II<sup>®</sup>; however, the data from year-to-year have been highly variable. As expected, the data have indicated that CEW larvae were more tolerant to the effects of MVP II<sup>®</sup> than TBW larvae. CEW and TBW populations have become more resistant to cypermethrin. In 2011, CEW populations were 19 times more resistant to cypermethrin than CEW populations sampled during the mid-1990s and 9 times more resistant to cypermethrin than CEW populations sampled during the mid-2000s. TBW populations collected during the 2011 season were on average 12 times more resistant than TBW populations sampled during the mid-1990s and ca. 3 times more resistant than TBW populations sampled during the mid-2000s. The data indicated that spinosad (Tracer<sup>®</sup>) has remained effective in the control of CEW and TBW populations in Georgia. There have been no substantial fluctuations in the activity of spinosad against CEW and TBW larvae throughout the study period. In general, the treated diet-96 h activity spectrum for the insecticides tested were as follows: CEW: Spinosad (Tracer<sup>®</sup>) > Cypermethrin > MVP II<sup>®</sup>; TBW: Spinosad (Tracer<sup>®</sup>) > MVP II<sup>®</sup> > Cypermethrin.

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