

LABORATORY EVALUATION OF SELECTED INSECTICIDES ON FIELD-COLLECTED POPULATIONS OF BOLLWORM AND TOBACCO BUDWORM LARVAE-2017 UPDATE

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Gregory Payne

Emily Adams

Department of Biology, University of West Georgia

Carrollton, GA 30118

Abstract

Bollworm (CEW; *Helicoverpa zea*) and tobacco budworm (TBW; *Heliothis virescens*) susceptibilities to MVP II®, cypermethrin, and spinosad (Tracer®) during the 2017 season. Results were compared to historical data collected throughout a twenty three-year study period, which began in 1995. Throughout the study period, the susceptibilities of CEW and TBW larvae to MVP II® have been highly variable. MVP II® LC₅₀ values for TBW were highest during the 2010 season; however, TBW larvae, MVP II® LC₅₀ values during the 2011-2016 seasons were some of the lowest recorded. Throughout the study period, TBW larvae, in Georgia, have remained susceptible to the effects of Cry1Ac, the *Bt* protein, found in MVP II®. On the other hand, MVP II® LC₅₀ values for populations of CEW collected during the past two seasons have been some of the highest values recorded during the study period. In 2016, MVP II® LC₅₀ values for field-collected CEW larvae ranged between 19.8 ppm and 527 ppm, with a mean LC₅₀ value of 213 ppm. In 2017, MVP II® LC₅₀ values for field-collected CEW larvae ranged between 88.4 ppm and 3941 ppm, with a mean LC₅₀ value of 1185 ppm, the highest mean LC₅₀ value recorded during the twenty three-year study period and 120-fold higher than the MVP II® LC₅₀ for the lab-maintained reference colony (BIO). Although cypermethrin remained an effective insecticide to control CEW larvae, average LC₅₀ values have fluctuated throughout the study period with the highest mean LC₅₀ value (26.8 ppm) being recorded in 2011. In 2017, field-collected CEW larvae were more susceptible, with a mean LC value comparable to mean LC₅₀ values obtained during the 2009-2010 seasons. In general, the effectiveness of cypermethrin for the control of TBW larvae has continued to decline throughout the study period. In 2017, the mean LC₅₀ value (194 ppm) for cypermethrin against TBW larvae was ca. 3-fold higher than the 2014-2015 LC₅₀ values and ca. 21-fold higher than the mean LC₅₀ values obtained during the 1990s (and ca. 92-fold higher as compared to a pyrethroid-susceptible BIO lab population). Data obtained from adult vial tests (AVT) conducted on CEW and TBW populations supported the larval studies and indicated that CEW and TBW populations in Georgia have become less susceptible to the effects of cypermethrin (pyrethroid insecticides). In 2017, % survivals of CEW and TBW at the 10 µg/vial rate were 39% and 81%, respectively. Spinosad (Tracer) has remained effective against CEW and TBW larvae throughout the study period. Although, the mean LC₅₀ value for spinosad (Tracer) against 2015 CEW larvae was the highest mean LC₅₀ value recorded during this study (ca. 13-fold higher than 1996 values and 6-fold higher than 2014 values), the 2016-2017 CEW and TBW mean LC₅₀ values for spinosad were similar to values previously observed. Prior to the 2015 season, the spinosad (Tracer) LC₅₀s for CEW and TBW larvae ranged between 0.30 ppm-1.34 ppm for CEW populations tested and between 0.20 ppm-0.84 ppm for TBW populations tested.

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, non-*Bt* corn, and chickpea fields throughout Georgia during the summer of 2017. Larvae from those field-collected samples were assayed for their susceptibility to a variety of insecticides using an insecticide treated-diet bioassay; adults were bioassayed for their susceptibility to cypermethrin using the adult vial test (AVT) method. Results were compared to baseline data collected between 1995-1999, 2003-2005, and 2009-2016.

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 2017 season, ten CEW and four TBW populations were collected from 7 counties including Carroll, Colquitt, Lowndes, Pike, Schley, Sumter, 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 (Southland Products, Inc., Lake Village, AR), 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® (19.1% A.I., Monsanto Corporation, St. Louis, MO), cypermethrin (94.3% A.I., FMC Corporation, Princeton, NJ), and spinosad (Tracer®; 91.3% A.I., Dow AgroSciences, Indianapolis, IN).

Larvae were evaluated using a modified insecticide-treated diet bioassay; adults were evaluated using an adult vial test (AVT) protocol. For the insecticide-treated diet bioassay, an insecticide test solution (100 µl) 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. Control diets were prepared using the solvent only. Distilled water was used as a solvent to prepare the MVP II test solutions; acetone was used to prepare the cypermethrin and spinosad (Tracer®) test solutions. The treated diet (1.0 mL) was distributed into each well of a 128-well bioassay tray (Bio-Quip Products, Inc., Rancho Dominguez, CA) and allowed to cool and gel. One neonate or one late 2nd instar larva (depending upon the insecticide being evaluated) was added to each well, and mortality was assessed following a 96 h exposure period. For the AVT bioassay, an insecticide test solution (500 µl) was added to a 20 mL borosilicate scintillation vial. Vials containing the insecticide solution were placed on a hotdog roller and continuously rolled until the solvent (acetone) had evaporated leaving a thin layer of insecticide coating the inside of the vial. Control vials were prepared with acetone alone. An individual moth was placed into each vial (1 moth/vial), and mortality was assessed following 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. Dose-response regressions were generated and analyzed using POLO probit analysis software (LeOra Software Company, Petaluma, CA).

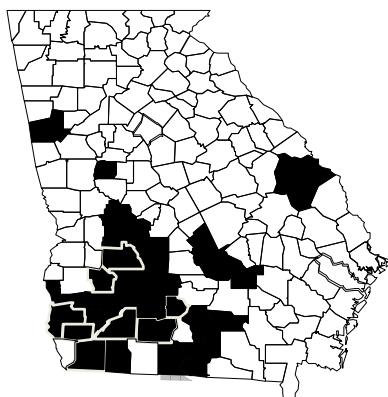


Figure 1. Cumulative bollworm and tobacco budworm collection sites---1995-2017.

Results and Discussion

As expected, MVP II® was less effective against CEW larvae as compared to TBW larvae (Tables 1-4; Figures 2 and 3). As in past seasons, CEW populations collected during the 2017 season exhibited substantially higher levels of survival following exposure to MVP II® as compared to TBW populations; average CEW LC₅₀ values for the 2017 season were ca. 1200-fold greater than average TBW LC₅₀ values obtained during the 2016 season. Although the susceptibilities of CEW and TBW larvae to MVP II® have been highly variable, the 2017 LC₅₀ values obtained for the populations tested within each species were some of the highest LC₅₀s recorded during the twenty three-year study period. The 2017 CEW mean LC₅₀ value was the highest mean value recorded since 2010 (Tables 1 and 2). The 2017 TBW mean MVP II® LC₅₀ was comparable to the 2015-2016 mean LC₅₀ values and among the lowest mean LC₅₀ values recorded since the study began in 1995. Variability in the susceptibilities of CEW and TBW larvae to MVP II® are an inherent issue and may be associated with the crop from which the populations were collected, the time during the season that the populations were collected, and exposure histories. However, more

than two thirds of the CEW populations tested had LC_{50} values greater than 600 ppm and one third of the CEW populations tested had LC_{50} values greater than 1000 ppm.

Decreases in the susceptibilities of CEW and TBW populations to pyrethroid insecticides have been noted throughout the study period. 2011 LC_{50} values (for both CEW and TBW) have been the highest LC_{50} s recorded to date (Tables 2 and 4; Figures 2-3) and averaged ca. 2-fold higher than the 2010 values. LC_{50} values for CEW populations collected during the 2011 season were more than 9-fold higher than the LC_{50} values for CEW populations collected during the 2005-2009 seasons and 19-fold higher than the LC_{50} values for CEW populations collected during the mid-1990s (Table 2; Figure 2). Although cypermethrin-treated diet bioassays were not conducted during the 2012-2013 seasons, adult vial tests conducted on 2012 populations of CEW confirmed that trend; the percent survival of 2012 populations of CEW adults was 2-fold greater at the 5 μ g cypermethrin/vial rate and 10-fold greater at the 10 μ g cypermethrin/vial rate than the percent survival of 2011 CEW adults (Figure 4). In recent years, treated diet LC_{50} values have indicated that CEW populations in Georgia had become more susceptible to the effects of cypermethrin. On average, the 2014 CEW populations were ca. 6-fold more susceptible than the 2011 populations with a mean LC_{50} value of 4.23 ppm (Table 2) and 2015 CEW populations were ca. 3-fold more susceptible than 2014 populations with a mean LC_{50} value of 1.44 ppm (Table 2). However, the 2015-2016 mean LC_{50} values were more than 4-fold greater than 2015. In treated diet bioassays conducted using TBW populations, the mean 2011 TBW LC_{50} value was ca. 1.4-fold higher than the mean 2009-2010 TBW LC_{50} value, ca. 3-fold higher than the mean 2003-2005 TBW LC_{50} value, and 12-fold greater than the mean 1995-1999 value (Table 4; Figure 3), and the lowest LC_{50} values recorded since 1995 were recorded in 2012 TBW populations (2012 mean LC_{50} = 2.9 ppm). However, successively higher mean LC_{50} values have been recorded during the last five seasons (Table 4; Figure 3) with the 2016-2017 mean LC_{50} values being the highest recorded to date. In general, AVT results were similar, in that steady increases in the percent survival of adult CEW and TBW have been noted since 1998 when AVT monitoring in my laboratory began (Figure 4).

To date, spinosad (Tracer[®]) has remained effective against all CEW and TBW populations tested. Of the three insecticides evaluated, spinosad has consistently been the most potent and efficacious (Tables 2 and 4; Figures 2 and 3; see past reports also). Since the introduction of Tracer[®] for field use in the mid-late 1990's, the susceptibilities of CEW and TBW larvae to spinosad have remained constant.

Summary

Throughout the twenty three-year study period:

- Bollworm (CEW) and tobacco budworm (TBW) populations in Georgia have remained relatively susceptible to MVP II[®]; 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[®] than TBW larvae. In general, the 2015-2017 CEW mean LC_{50} values (161, 213, and 1184 ppm, respectively) have been some of the highest mean LC_{50} values recorded to date, and LC_{50} values for more than half of the 2016 CEW populations tested were greater than 200 ppm; LC_{50} values for ca. two thirds of the 2017 CEW populations tested were greater than 600 ppm; and LC_{50} values for ca. one third of the 2017 CEW populations tested were greater than 1000 ppm;
- CEW and TBW populations have become more resistant to cypermethrin, and decreases in the susceptibilities of CEW and TBW populations to cypermethrin using a treated diet bioassay have been confirmed by AVT. Although pyrethroid resistant CEW and TBW populations remain a major concern for growers across the state of Georgia and throughout the southeast, data collected by this lab during the 2012-2015 seasons seemed to have indicated that the levels of pyrethroid resistance in CEW and TBW populations collected from across the state had stabilized and that CEW populations had become slightly more susceptible to the effects of cypermethrin. However, the mean 2016-2017 CEW LC_{50} values were the highest since 2010, and the mean 2016-2107 TBW LC_{50} values have been the highest recorded during this study.
- Data collected from 1995 through 2015 indicated that spinosad (Tracer[®]) has remained effective in the control of CEW and TBW populations in Georgia.
- In general, the treated diet activity spectra of the insecticides tested against CEW and TBW populations (Figures 2 and 3) remained as follows:

CEW: Spinosad (Tracer[®]) > Cypermethrin > MVP II[®]
 TBW: MVP II[®] ≥ Spinosad (Tracer[®]) > Cypermethrin

- Continued monitoring of the susceptibilities of MVP II[®], pyrethroids, and spinosad (Tracer[®]) is recommended. Evaluations should be expanded to include additional *Bt* proteins (e.g., Cry2Ab, Cry2Ae, Cry1Ab, VIP 3A,...) and chlorantraniliprole; however, the availability of the *Bt* proteins (especially purified) have been limited. Although efforts to increase the success of CEW evaluations during the upcoming seasons should remain a priority, monitoring the effects of these insecticides against TBW should continue. This lab is one of the few labs across the southeast and mid-south that has made an effort to continuously monitor the susceptibilities of CEW and TBW populations to the principle insecticides used for their control.

Acknowledgements

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Table 1. Susceptibilities of field-collected bollworm (CEW) larvae to MVP II[®], cypermethrin, and spinosad (Tracer[®]) using a treated diet bioassay-2017.

Treatment	Colony	County	N	LC ₅₀ ppm	95% C.I.	Slope (S.E.)	χ^2	df
MVP II [®]	BIO Lab		877	9.831	6.346-15.01	1.718 (0.109)	1.903	6
	CAR 1	Carroll	630	143.7	98.02-224.2	0.812 (0.069)	3.670	6
	CAR 2	Carroll	577	1149	674.3-2493	0.940 (0.116)	5.267	6
	LOW	Lowndes	426	777.3	399.7-2055	0.725 (0.094)	1.256	6
	PIK	Pike	723	88.38	48.71-182.3	0.726 (0.058)	10.56	6
	SCH	Schley	610	3310	1466-11901	0.956 (0.157)	1.952	6
	SUM 1	Sumter	458	749.0	358.9-2215	0.629 (0.081)	4.571	6
	SUM 2	Sumter	597	1222	733.5-2619	1.020 (0.134)	2.115	6
	TIF 1	Tift	607	3941	1416-21276	0.553 (0.078)	5.917	6
	TIF 2	Tift	429	102.1	58.71-198.3	0.804 (0.081)	6.302	6
	TIF 3	Tift	713	705.6	580.3-901.3	3.235 (0.459)	4.395	6
	Texas		284	632.8	349.1-1574	0.979 (0.156)	2.574	6
	Low LC₅₀	88.38						
	High LC₅₀	3941						
	Mean LC₅₀	1185						

Table 1 (Continued). Susceptibilities of field-collected bollworm (CEW) larvae to MVP II[®], cypermethrin, and spinosad (Tracer[®]) using a treated diet bioassay-2017.

Treatment	Colony	County	N	LC ₅₀	95% C.I.	Slope (S.E.)	χ^2	df
Cypermethrin	BIO Lab		698	1.194	0.6471-2.231	3.861 (0.319)	2.032	6
	CAR 1	Carroll	76	2.896	1.976-4.183	4.171 (1.121)	0.467	6
	CAR 2	Carroll	466	4.439	3.351-5.865	2.749 (0.237)	11.57	6
	LOW	Lowndes	604	5.523	4.805-6.362	2.742 (0.213)	5.862	6
	PIK	Pike	157	5.527	3.316-9.598	2.914 (0.448)	10.84	6
	SCH	Schley	269	5.065	4.115-6.258	2.575 (0.279)	4.716	6
	SUM 2	Sumter	284	6.131	5.012-7.491	2.783 (0.314)	3.839	6
	TIF 1	Tift	119	7.445	5.721-9.767	4.461 (0.968)	0.617	6
	TIF 3	Tift	295	9.180	7.812-10.75	4.407 (0.602)	2.967	6
	Texas		147	>50				
	Low LC ₅₀	2.896						
	High LC ₅₀	9.180						
	Mean LC ₅₀	5.776						

Table 1 (Continued). Susceptibilities of field-collected bollworm (CEW) larvae to MVP II[®], cypermethrin, and spinosad (Tracer[®]) using a treated diet bioassay-2017.

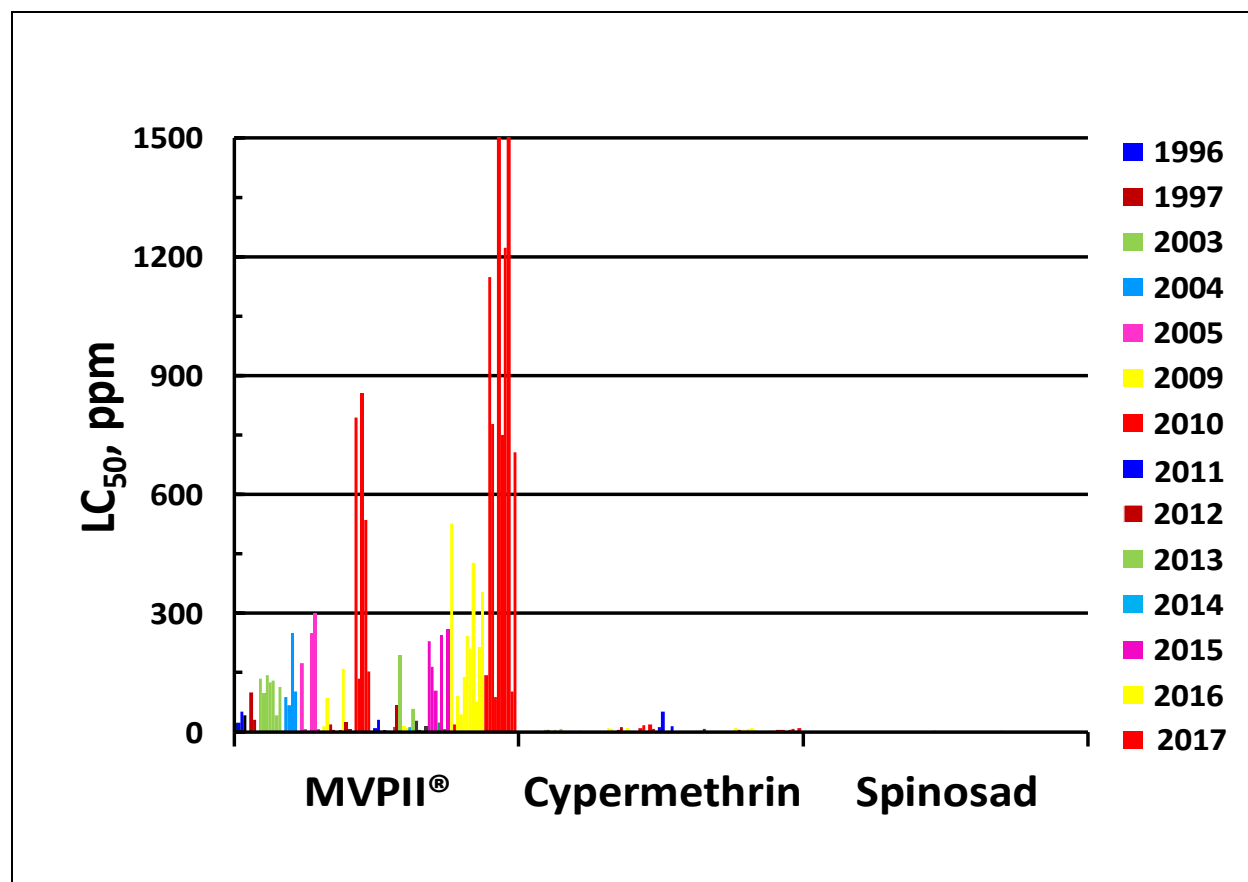
Treatment	Colony	County	N	LC ₅₀	95% C.I.	Slope (S.E.)	χ^2	df
Spinosad/ Tracer[®]	BIO Lab		445	0.269	0.212-0.341	2.202 (0.278)	4.526	6
	CAR 1	Carroll	164	0.474	0.362-0.621	3.738 (0.689)	0.268	5
	CAR 2	Carroll	224	0.863	0.649-1.132	3.521 (0.663)	0.358	6
	LOW	Lowndes	303	0.503	0.412-0.619	5.146 (0.847)	0.006	6
	SCH	Schley	388	0.666	0.534-0.829	2.812 (0.335)	0.825	6
	SUM 2	Sumter	160	0.390	0.269-0.583	2.827 (0.551)	5.126	6
	TIF 1	Tift	93	0.931	0.576-1.506	2.782 (0.669)	0.093	6
	TIF 2	Tift	41	0.295	0.135-0.590	2.062 (0.600)	1.160	4
	TIF 3	Tift	288	0.346	0.269-0.449	2.942 (0.421)	2.134	6
	Low LC₅₀	0.295						
	High LC₅₀	0.931						
	Mean LC₅₀	0.558						

Table 2. Mean susceptibilities of bollworm larvae to MVPH[®], cypermethrin and spinosad (Tracer[®]) following a 96 h exposure period using an insecticide-treated diet bioassay--1996-2017.

Year	Average LC ₅₀ , ppm (Slope)		
	MVPH [®]	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)
2012	41.3 (1.2)*	ND	ND
2013	72.9 (1.7)*	ND	ND
2014	13.2 (1.1)*	4.23 (3.6)	0.66 (1.9)
2015	161 (1.4)*	1.44 (3.3)	3.92 (2.5)
2016	213 (1.0)*	7.04 (3.3)	0.74 (2.7)
2017	1185 (1.0)*	5.78 (3.4)	0.56 (3.2)

ND = Not Determined

* Data based on tests using neonate larvae



Potency: Spinosad > Cypermethrin > MVP II®

Figure 2. Susceptibilities of field-collected bollworm larvae to MVP II®, spinosad (Tracer®), and cypermethrin using a treated diet bioassay—1996-2017.

Table 3. Susceptibilities of field-collected tobacco budworm (TBW) larvae to MVP II[®], cypermethrin, and spinosad (Tracer[®]) using a treated diet bioassay-2017.

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Table 3 (Continued). Susceptibilities of field-collected tobacco budworm (TBW) larvae to MVP II[®], cypermethrin, and spinosad (Tracer[®]) using a treated diet bioassay-2017.

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Table 3 (Continued). Susceptibilities of field-collected tobacco budworm (TBW) larvae to MVP II[®], cypermethrin, and spinosad (Tracer[®]) using a treated diet bioassay-2017.

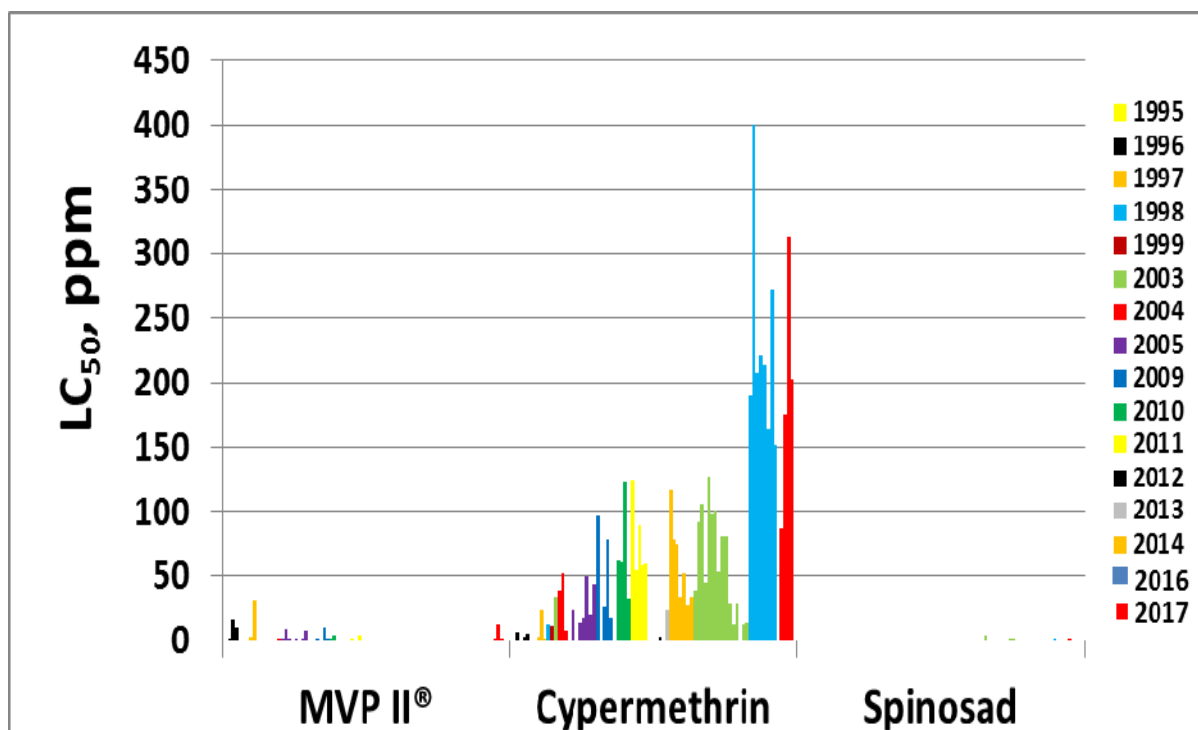
Treatment	Colony	County	N	LC ₅₀	95% C.I.	Slope (S.E.)	χ^2	df
Spinosad/Tracer[®]	CAR	Carroll	321	1.697	1.291-2.237	2.462 (0.337)	2.952	6
	COL	Colquitt	242	1.126	0.403-3.450	1.683 (0.199)	20.26*	6
	LOW	Lowndes	160	0.377	0.241-0.588	1.912 (0.315)	1.496	6
	TIF	Tift	304	0.862	0.498-1.549	1.972 (0.236)	11.57	6
	Low LC₅₀	0.377						
	High LC₅₀	1.697						
	Mean LC₅₀	1.016						
* The χ^2 (df) value followed by “***” indicated poor fit of the data to the probit model (P>0.05).								

Table 4. Mean susceptibilities of tobacco budworm larvae to MVP[®], cypermethrin and spinosad (Tracer[®]) following a 96 h exposure period using an insecticide-treated diet bioassay--1995-2017.

Strain	LC ₅₀ , ppm (Slope)		
	MVP [®]	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)
2012	0.13 (1.7)*	2.9 (1.9)	ND
2013	ND	22.9 (2.6)	ND
2014	0.29 (1.5)*	59.5 (2.3)	0.46 (2.0)
2015	0.12 (2.0)*	59.4 (2.5)	0.73 (1.5)
2016	0.18 (1.2)*	228 (2.1)	0.55 (2.3)
2017	3.90 (1.0)*	194 (2.7)	1.02 (2.0)

ND = Not Determined; HRV = pyrethroid-susceptible lab colony; OPS = organophosphate- and pyrethroid-susceptible lab colony; OPR = organophosphate-resistant and pyrethroid-susceptible lab colony; PYR = laboratory-selected, pyrethroid-resistant lab colony

* Data based on tests using neonate larvae



Potency: MVP II® ≥ Spinosad > Cypermethrin

Figure 3. Susceptibilities of field-collected tobacco budworm larvae to MVP II®, spinosad (Tracer®), and cypermethrin using a treated diet bioassay—1996-2017.

Table 5. Susceptibilities of bollworm (CEW) and tobacco budworm (TBW) adults to cypermethrin using an adult vial test (AVT) bioassay—1998-2017.

Treatment	Species	Rate, µg/vial	% Survival*
Cypermethrin	CEW	5	58
		10	39
		20	14
	TBW	5	91
		10	81
		20	70
*0% survival following a 24h exposure period			

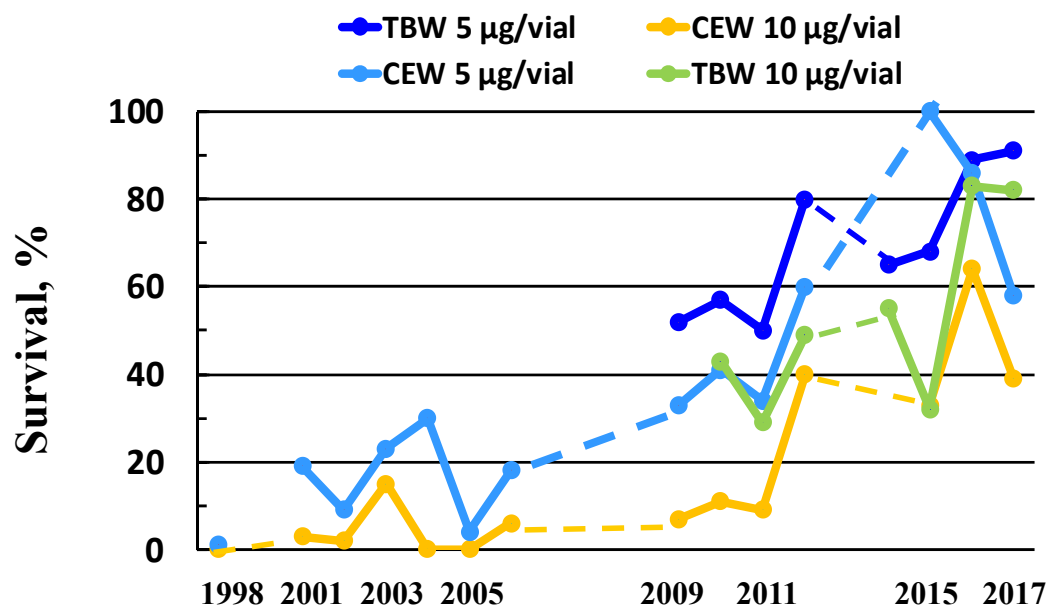


Figure 4. Susceptibilities of bollworm (CEW) and tobacco budworm (TBW) adults to cypermethrin using an adult vial test (AVT) bioassay—1998-2017.