# INSECTICIDE SUSCEPTIBILITY OF LOUISIANA BOLLWORM AND TOBACCO BUDWORM POPULATIONS D.R. Cook, B.R. Leonard, R.D. Bagwell, S. Micinski, J. Gore, R.H. Gable and A.M. Stewart Louisiana State University Agricultural Center Baton Rouge, LA

#### <u>Abstract</u>

Twenty-five pairs of pheromone-baited wire cone traps representing 12 parishes in Louisiana were used to survey insecticide susceptibility of tobacco budworm and bollworm from May to Sep during 2001. Percent survival (10µg/vial cypermethrin in AVT) of tobacco budworm moths ranged from 47% to 76%, with a mean survival of 59%. Percent survival (5µg/vial cypermethrin in AVT) for bollworm moths ranged from 6% to 32%, with a mean survival of 21%. No field control failures of bollworm with pyrethroids were reported in Louisiana during 2001. Susceptibility of tobacco budworm and bollworm to spinosad also was monitored using the AVT (5µg/vial and 15µg/vial concentrations). Tobacco budworm survival ranged from 4% to 16% and 0% to 19% for the 5µg/vial and 15µg/vial concentrations, respectively. Higher than expected survival (31%-56% at 15µg/vial) of male tobacco budworm moths was observed at several locations in Northeast Louisiana. Bollworm survival ranged from 0% to 40% and 0% to 28% for the 5µg/vial and 15µg/vial concentrations, respectively. Suspected field control failures of infestations of tobacco budworm larvae following applications of Tracer 4SC (spinosad) were reported in Northeast Louisiana during 2001. These problems coincided with locations where high survival of male tobacco budworm larvae following applications from these areas indicate elevated LD<sub>50</sub> and LD<sub>90</sub> values and changes in slopes of log-dose probit lines compared to reference colonies.

# **Introduction**

Insecticide resistance in key insect pests is an important issue for the cotton industry. Two of the most important cotton pests in the Mid-South and Southeastern United States are the tobacco budworm, *Heliothis virescens* (F.), and the bollworm, *Helicoverpa zea* (Boddie). Resistance to organochlorines, DDT, organophosphates (Sparks 1981), and carbamates (Elzen et al. 1992) has been reported with both species. Resistance in tobacco budworm populations to pyrethroid insecticides was documented in Arkansas (Plapp et al. 1987), Louisiana (Leonard et al. 1987), Mississippi (Roush and Luttrell 1987), and Texas (Allen et al. 1987, Plapp et al. 1987) during 1986. In response, pyrethroid resistance management plans were implemented in those states (Anonymous 1986). An important component of these plans involves systematic insecticide resistance monitoring of male tobacco budworm moths using the adult vial test (AVT) described by Plapp et al. (1987).

In Louisiana, pyrethroid (10µg/vial cypermethrin) resistance in tobacco budworm populations increased from 1986 to 1993 (Graves et al. 1988, 1989, 1990, 1991, 1992, 1993, 1994), peaking at a mean survival of 48% for the period of May through Aug in 1993. Resistance levels stabilized from 1994 to 1996 with a mean survival of 39% (Bagwell et al. 1995, 1996, 1997). However, pyrethroid resistance again increased to 55% survival in 1997 and 61% in 2000 (Bagwell et al. 1998, 1999, 2000, 2001). In response to the high levels of resistance in tobacco budworm populations, pyrethroids are not recommended for tobacco budworm control in Louisiana.

Pyrethroid resistance monitoring of bollworm populations began in Louisiana during 1988. Mean bollworm survival to a  $5\mu$ g/vial concentration of cypermethrin has increased from 2% in 1988 to 16% in 2000 (Graves et al. 1988, 1989, 1990, 1991, 1992, 1993, 1994, Bagwell et al. 1995, 1996, 1997, 1998, 1999, 2000, 2001). Resistance to DDT and organochlorine insecticides has been reported in bollworm (Sparks 1981). Also, field control failures (Walker et al. 1998) resulting from pyrethroid-resistant populations of bollworm (Brown et al. 1998) have been reported in South Carolina. In most cotton production areas, organophosphates, carbamates, and pyrethroids have generally remained effective against bollworm (Kharboutli et al. 1999, Brickle et al. 2000, Bagwell et al. 2001).

Monitoring of tobacco budworm and bollworm susceptibility to spinosad began during 1991 and 2000 in Louisiana, respectively. Bollworm and tobacco budworm moths were monitored from Jul to Aug using  $5\mu g$  and  $15\mu g$  concentrations of spinosad in the AVT. In 2000, the mean tobacco budworm survival was 48% and 21% for the  $5\mu g$ /vial and  $15\mu g$ /vial concentrations of spinosad, respectively (Cook et al. 2001). Mean survival of bollworm moths was 54% at the  $5\mu g$ /vial concentration and 19% at the  $15\mu g$ /vial concentration (Cook et al. 2001).

This report summarizes the results of monitoring tobacco budworm and bollworm populations exposed to cypermethrin and spinosad with the AVT. Suspected field control failures associated with Tracer applications were reported in localized areas of Northeast Louisiana during the 2001 growing season. In response to these observations, collections from these fields also

were tested for susceptibility to spinosad. The results were compared to that of 1991 field collections and a laboratory reference colony.

# **Materials and Methods**

Adult vial bioassays (AVT) similar to those described by Plapp et al. (1987, 1990) were utilized to monitor the susceptibility of field collected tobacco budworm and bollworm moths to cypermethrin and spinosad (Tracer 4SC, Dow Agrosciences, 9330 Zionsville Rd., Indianapolis, IN 46268). Stock solutions of cypermethrin and spinosad were made by dissolving technical grade insecticide in acetone. Serial dilutions were made from each stock solution to yield the desired insecticide concentrations. The interior surface of 20 ml glass scintillation vials was coated with insecticide by pipetting 0.5 ml of the appropriate insecticide solution into the vials. These vials were rotated on a modified hot dog roller (heating element disconnected) until all of the acetone had evaporated. Vials were stored in a dark environment until used.

Male tobacco budworm and bollworm moths were collected using wire cone traps (Hartstack et al. 1979) baited with artificial sex pheromone lures (Hendricks et al. 1987) from May through Sep. Moths were collected from 12 parishes within the cotton production regions of Louisiana. In addition, more intensive sampling was conducted at or near the Northeast Research Station (Macon Ridge location, Franklin Parish), the Red River Research Station (Bossier Parish), and the Dean Lee Research Station (Rapides Parish). The insecticide concentrations used in these monitoring efforts included 10 $\mu$ g/vial cypermethrin for tobacco budworm, 5 $\mu$ g/vial cypermethrin for bollworm and 5 $\mu$ g/vial and 15 $\mu$ g/vial spinosad for both tobacco budworm. Moths were placed into insecticide-treated and control (non-treated) vials (1 moth/vial) and mortality was determined after 24-h of exposure (HAE). Only moths that appeared healthy were used for testing. Moths were considered dead if they were incapable of sustained flight for 3 ft. Data were corrected for control mortality using Abbott's (1925) formula.

Tobacco budworm larvae were collected from fields with suspected field control failures following Tracer 4SC (spinosad) applications and placed into 30 ml plastic cups (Solo Cup Company, Urbana, IL 61801) containing meridic diet described by Shour and Sparks (1981). Field collected insects were allowed to complete one generation in the laboratory to achieve adequate numbers of larvae for laboratory bioassays. Technical grade spinosad was dissolved in acetone, and serial dilutions were used to yield the desired concentrations. One µl of solution was applied to the thoracic dorsum of each larva using a Hamilton microsyringe. Larvae in the control treatment were treated with 1µl of acetone. A minimum of 40 larvae (larval weight 15-25mg) per dose was utilized in all bioassays. Mortality was determined at 48 and 72 hours after treatment. Larvae were considered dead if they could not right themselves after being rolled onto their dorsal surface. Data were corrected for control mortality (Abbott 1925) and analyzed by probit analysis using Polo PC (LeOra Software 1987). Reference colonies utilized in this study included the LSU tobacco budworm laboratory colony which has been in continuous culture since 1977 (Leonard et al. 1987), as well as, larvae from the F2 generation of a colony established from velvetleaf at the Macon Ridge location of the Northeast Research Station during May 2001. The 1991 field data is from collections made from cotton fields in Louisiana, Mississippi, and Texas (J.B. Graves; LSU AgCenter, retired, and S. H. Martin, Syngenta, Unpublished Data).

# **Results and Discussion**

In 2001, 25 pairs of pheromone baited wire cone traps were distributed across the cotton production areas (12 parishes) in Louisiana to survey species composition of tobacco budworm and bollworm. Over 700 tobacco budworm moths were assayed for pyrethroid susceptibility from May to Sep 2001. Percent survival in May, Jun, Jul, Aug, and Sep was 69%, 47%, 73%, 76%, and 57%, respectively, with an average survival of 59% (Table 1). Tobacco budworm survival during May, Jul, and Aug was the highest observed for the respective months since resistance monitoring was initiated in Louisiana. Moth survival during Aug (76%) was the highest monthly average survival that has been observed in Louisiana. Average survival for the entire season was similar to that observed during 1999 and 2000. These data further indicate that pyrethroids are no longer a viable option for tobacco budworm control in Louisiana and justify their removal from Louisiana Cooperative Extension Service recommendations.

Over 800 bollworm moths were assayed for pyrethroid susceptibility from May to Sep 2001. Percent survival for May, Jun, Jul, Aug, and Sep was 10%, 6%, 32%, 13%, and 16%, respectively, with a mean survival of 21% (Table 2). Survival observed during May, Jun, and Aug was similar to or lower than survival observed during 1998 through 2000. Bollworm survival (32%) during Jul was the highest observed for the  $5\mu$ g/vial concentration of cypermethrin since the initiation of bollworm resistance monitoring in Louisiana. However, no field control failures of bollworm with pyrethroids were reported in Louisiana during 2001. Pyrethroids remain the most cost-effective insecticides for bollworm control in cotton.

Tobacco budworm survival (423 insects tested) for the 5µg/vial concentration of spinosad during May, Jun, Jul, Aug, and Sep was 4%, 9%, 15%, 19%, and 12%, respectively with an average survival of 11% (Table 3). Tobacco budworm survival at the

 $5\mu g$  concentration during Jul, Aug, Sep, and the season average was lower than that observed for the respective periods during 2000. Survival (781 insects tested) at the  $15\mu g$ /vial concentration during May, Jun, Jul, Aug, and Sep was 0%, 1%, 19%, 17%, and 0%, respectively (Table 3). Tobacco budworm survival at the  $15\mu g$  concentration was higher during Jul 2001 compared to Jul 2000. The average survival at  $15\mu g$  was 13% and was lower than that observed during 2000. The variation in summarized data between the  $5\mu g$  and  $15\mu g$  concentrations is a result of the  $5\mu g$ /vial concentration not being utilized at some locations. Higher than expected survival (31%-56% at  $15\mu g$ /vial) of male tobacco budworm moths was observed at several locations in Northeast Louisiana.

Bollworm survival (310 insects tested) for the  $5\mu$ g/vial concentration of spinosad during May, Jun, Jul, Aug, and Sep was 0%, 4%, 40%, 7%, and 3%, respectively, with an average survival of 11% (Table 4). Survival was lower during Jul and Aug compared to 2000. Bollworm survival (311 insects tested) at the  $15\mu$ g/vial concentration during May, Jun, Jul, Aug, and Sep was 0%, 0%, 28%, 3%, and 0%, respectively, with an average survival of 10% (Table 4). Survival during Jul (28%) was higher than that observed during Jul 2000.

Suspected field control failures of infestations of tobacco budworm larvae following applications of Tracer 4SC (spinosad) occurred in Northeast Louisiana during 2001. These problems coincided with some locations where high survival of male tobacco budworm moths to spinosad was observed in the AVT. The Giltner swamp, Bayou Macon, and Wollerson colonies had significantly higher  $LD_{50}$  values compared to the velvetleaf (pre-season field reference) and LSU Lab 2001 (laboratory reference) colonies based on non overlap of the 95% confidence limits (Table 5). The  $LD_{50}$  values for the Eckard road and Larto colonies were significantly higher than that for the LSU Lab 2001 colony, but was similar to the velvetleaf colony. Also, the  $LD_{50}$  value of the Giltner Swamp colony was significantly higher than those for the Red River Research Station and Clarksdale, MS 1991 field colonies. The  $LD_{50}$  values for all of the 1991 field colonies were similar to the LSU Lab 2001 colony was higher than that for the LSU Lab 2001 colonies were similar to the LD<sub>50</sub> value of the Giltner Swamp colony was significantly higher than those for the Red River Research Station and Clarksdale, MS 1991 field colonies. The  $LD_{50}$  values for all of the 1991 field colonies were similar to the LSU Lab 2001 colony was higher than that for the LSU Lab 2001 colony. Also, the slopes of the log dose probit lines for these colonies was higher than that for the LSU Lab 2001 colony indicating a greater degree of homogeneity in response of individuals in the populations.

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Table 1.	Summary	of cypermethrin	(10µg/vial)	resistance	monitoring	data	for t	obacco	budworm	male	moths,
1987-200	1.										

_	Month – Percent Survival (Number Tested)							
Year	May	Jun	Jul	Aug	Sep	Total		
1987	20	13	18	12	15	15 (2607)		
1988	12	5	14	26	30	16 (2214)		
1989	11	9	19	40	36	25 (3057)		
1990	12	14	36	43	48	37 (3605)		
1991	16	21	31	42	49	36 (3539)		
1992	14	22	39	58	56	40 (4281)		
1993	22	29	46	58	68	48 (2823)		
1994	20	26	44	50	59	39 (1716)		
1995	18	20	37	49	49	39 (2131)		
1996	23	24	50	43	-	39 (1966)		
1997	26	51	51	63	-	55 (745)		
1998	41	48	51	60	-	50 (769)		
1999	46	64	53	58	-	60 (509)		
2000	62	22	43	63	-	61 (686)		
2001	69	47	73	76	57	59 (728)		

Data prior to 2001 summarized in Bagwell et al. (2001).

	Month – Percent Survival (Number Tested)								
Year	Conc.	May	Jun	Jul	Aug	Sep	Total		
1988	1μg	10	0	64	34	30	43 (515)		
	2µg	0	-	7	15	20	13 (253)		
	5µg	0	0	3	2	3	2 (439)		
1989	1μg	-	-	57	60	38	53 (220)		
	2µg	-	-	49	48	30	43 (220)		
	5µg	-	-	5	6	3	4 (170)		
1990	1μg	19	33	44	34	24	33 (1064)		
	2μg	5	25	28	16	15	21 (1040)		
	5µg	0	0	6	1	2	2 (561)		
1991	1µg	25	54	50	43	37	44 (1909)		
	2μg	11	23	31	23	26	24 (1830)		
	5µg	2	5	7	4	8	5 (1666)		
1992	1µg	31	32	55	45	46	42 (1241)		
	2μg	24	19	41	34	19	31 (1295)		
	5µg	3	2	11	7	12	8 (932)		
1993	1µg	-	22	53	50	55	49 (530)		
	2μg	-	21	36	30	48	33 (733)		
	5µg	-	0	7	7	9	7 (483)		
1994	1µg	37	50	60	56	-	55 (643)		
	2μg	27	33	45	42	-	40 (683)		
	5µg	3	9	10	8	-	8 (500)		
1995	1µg	53	40	67	58	-	59 (773)		
	2μg	20	23	45	38	-	36 (767)		
	5µg	3	0	8	7	-	6 (580)		
1996	5µg	4	3	9	5	-	7 (3697)		
1997	5µg	4	4	14	7	-	9 (1821)		
1998	5µg	12	14	27	19	-	18 (1950)		
1999	5µg	18	13	15	16	-	16 (809)		
2000	5µg	13	20	19	14	-	16 (1445)		
2001	5µg	10	6	32	13	16	21 (829)		

Table 2. Summary of cypermethrin  $(1, 2, and 5\mu g per vial)$  resistance monitoring data for male bollworm moths, 1988-2001.

Data prior to 2001 summarized in Bagwell et al. (2001).

	Month - Percent Survival (Number Tested)							
Year	Conc.	May	Jun	Jul	Aug	Sep	Total	
1991	5µg	-	-	-	5	4	5 (390)	
	15µg	-	-	-	3	3	3 (295)	
1992	5µg	-	-	6	-	-	6 (200)	
	15µg	-	-	0.5	-	-	0.5 (200)	
1993	5µg	-	-	-	7	18	13 (262)	
	15µg	-	-	-	2	1	1 (262)	
2000	5µg	-	-	21	51	66	48 (436)	
	15µg	-	-	8	18	38	21 (429)	
2001	5µg	4	9	15	19	12	11 (423)	
	15µg	0	1	19	17	0	13 (781)	

Table 3. Responses of field-collected male tobacco budworm moths to spinosad in AVT, 1991-1993 and 2000-2001.

Data prior to 2001 summarized in Cook et al. (2001).

Table 4.	Responses of	field-collected	i male bollworm	n moths to si	binosad in	AVT.	2000-2001
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	Month - Percent Survival (Number Tested)								
Year	Conc.	May	Jun	Jul	Aug	Sep	Total		
2000	5µg	-	-	52	58	-	54 (149)		
	15µg	-	-	26	5	-	19 (145)		
2001	5µg	0	4	40	7	3	11 (310)		
	15µg	0	0	28	3	0	10 (311)		

Data prior to 2001 summarized in Cook et al. (2001).

Table 5. Responses of tobacco budworm larvae from field and laboratory colonies to topical applications of spinosad.

Colony	$LD_{50}^{1}$	95% C.L.	Slope±S.E.
1991			•
Red River Research Stn. <sup>2</sup>	0.036	0.019-0.059	$1.06\pm0.19$
Clarksdale,MS <sup>2</sup>	0.036	0.020-0.057	$1.53\pm0.31$
Uvalde, TX <sup>2</sup>	0.043	0.016-0.082	$0.84\pm0.17$
2001			
LSU Lab 2001 (lab reference)	0.009	0.004-0.018	$0.68\pm0.07$
Velvetleaf (pre-season field reference)	0.029	0.011-0.048	$1.14\pm0.18$
Eckard Rd.	0.078	0.038-0.138	$0.82\pm0.15$
Giltner Swamp	0.087	0.063-0.114	$1.21\pm0.17$
Bayou Macon	0.103	0.051-0.178	$1.07\pm0.16$
Wollerson	0.101	0.056-0.163	$1.38\pm0.17$
Larto	0.05	0.029-0.073	$1.17\pm0.15$

 $^{1}\mu g$ /larva.  $^{2}$ Dr. J. B. Graves, LSU AgCenter, retired and Dr. S. H. Martin, Syngenta, Unpublished Data.