#### 2010 PYRETHROID RESISTANCE MONITORING OF BOLLWORMS

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### <u>Abstract</u>

Polyphagous bollworms are potentially exposed to pyrethroid insecticides during each generation. Since cotton is a host during the latter part of the growing season, any resistance developed during the season will reduce control realized in cotton. Pheromone traps have been used sporadically since the late 1980s throughout the cotton belt to collect male moths for testing resistance to a pyrethroid insecticide. Testing continued during 2010 using a concentration of 5  $\mu$ g/vial of cypermethrin as the primary diagnostic dose. Overall survival during 2010 was 18.1%, which was similar to the previous two years. However, comparisons of survival from 1998-200 with 2008-2010 show that overall survival at 5  $\mu$ g/vial has increased from 8% to 18%. Consistent with historical data, survival is highest during July and August when most moths are emerging from grass hosts.

### **Introduction**

Bollworm, *Helicoverpa zea*, is a pest in numerous crops where it may be controlled by the application of a pyrethroid insecticide. Since it can have 5 or more generations per year in the southern U.S., it has the potential to develop large populations. Furthermore it is very mobile, so resistance developing in one habitat or location can quickly spread to many regions. Transgenic Bt cotton provides some control, but foliar pyrethroid applications in

cotton targeting the bollworm are still common (Williams 2010). Therefore, an awareness of any developments of pyrethroid resistance would have a significant impact on current pest management practices.

Monitoring pyrethroid resistance in bollworms has been conducted for numerous years, beginning in 1988 in a few states and then coordinated throughout the cotton belt in 1989-1990. Since then monitoring has continued at various levels every year. Throughout this time the methodology has remained consistent. Male moths are captured in a pheromone trap and placed in a glass vial that was previously treated with insecticide. Mortality is recorded after 24 h. A concentration of 5  $\mu$ g cypermethrin/vial has been used with baseline survival generally less than 10% (Martin et al. 1999). However, over the last few years Louisiana has shown survival of up to 40% at the 5  $\mu$ g/vial dose (Leonard, B.R., unpublished data). Some data has also been collected using a concentration of 10  $\mu$ g cypermethrin/vial.

In addition to the adult vial test, the <sup>13</sup>C:<sup>12</sup>C ratios in the wings of the moths were also tested. The ratios in insect wings have been found to mirror the host plant on which the larva developed (Gould et al. 2002). Plants that have a  $C_3$  photosynthetic pathway (primarily broadleaves) have a different <sup>13</sup>C:<sup>12</sup>C ratio than plants with a  $C_4$  pathway (primarily grasses). Therefore this test allows us to know whether the moth had developed on a grass or broadleaf host. By pairing this information with the pyrethroid resistance, we can tell if the larval host differs between the pyrethroid-resistant and susceptible moths.

### **Materials and Methods**

Hartstack pheromone traps were placed in various locations in nine states across the cotton belt from VA to TX. Pheromones (Luretape with Zealure, Hercon Environmental) were changed every 2 weeks. Some traps were monitored at least weekly from April until October, but most were monitored over a shorter period when cotton was susceptible to bollworm feeding. Healthy moths caught in these traps were subsequently tested for pyrethroid resistance. Moths were individually placed in 20 ml scintillation vials that had been previously coated with 0 or 5  $\mu$ g cypermethrin per vial. Moths were kept in the vials for 24 h and then checked for mortality. Moths were considered dead if they could no longer fly. Moths that could fly from the 5  $\mu$ g cypermethrin vials (resistant moths) were recaptured and placed in a freezer along with a random subset of susceptible moths. All frozen moths were shipped to USDA-ARS in Stoneville, MS for carbon isotope analysis. Reported survival was corrected for control mortality (Abbott 1925). Vials were prepared in three laboratories and shipped to other locations as needed to verify results. Cross-checking between laboratories showed consistency in results.

To process moths for carbon isotope analysis, moths were removed from the freezer, and the left forewing from each moth was cut into small pieces. These wing pieces were placed into a 5x9mm tin capsule that was tightly folded into a cube. Wing tissue within each tin capsule was converted to  $CO_2$  by micro-Dumas combustion using a Costech ECS4010 Elemental Analyzer coupled to a Thermo Finnigan Delta plus Advantage Mass Spectrometer using a Conflo II Interface. Various isotope standard reference materials were used including acetanilide, urea, caffeine, and lyophilized corn tissue powder. Moths with  $\delta^{13}$ C values between -14 and -7 units per mil (%*c*) were considered to have a  $C_4$  host signature, whereas moths with  $\delta^{13}$ C values between -28 and -20%*c* developed on  $C_3$  hosts. Results from the analysis were used to distinguish between insects that developed on a  $C_4$  host (primarily grasses) from those that developed on a  $C_3$  host (primarily broadleaves). Isotope analysis has been conducted on moths collected in 2007 and 2008.

# **Results and Discussion**

Over all sites in the 9 participating states throughout 2010, more than 16,000 moths were tested, and 13.5% survived a concentration of 5 µg cypermethrin/vial. This varied widely by state and by month of year. Survival was highest overall in July with 18.1% survival which was consistent with data from previous years (Figure 1) Louisiana had the highest survival rate in every month of monitoring as it has in recent years. There has been no overall change in pyrethroid susceptibility within the last three years of monitoring, although individual states have shown variation. A more consistent trend emerges when viewed on a longer time scale. The last three years of monitoring data (2008-2010) were compared to data from 10 years earlier (1998-2000) as reported by Martin et al (1999, 2000) and Payne et al. (2001). Over this decade, overall survival during July increased from 8.2% to 18.2%. However the changes varied from minimal change in Missouri, South Carolina and Texas to increases of 35.3% and 21.7% in Louisiana and Virginia, respectively (Table 1).

Most larvae tested during July developed on  $C_4$  hosts, many presumably from corn. Comparisons between larval hosts of resistant and susceptible moths have not yet revealed any significant differences.

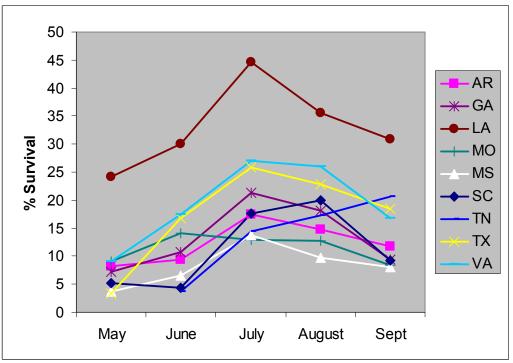


Figure 1. Average bollworm survival in the adult vial test at by month at 5 µg cypermethrin/vial during 2007-2010.

Table 1. Average bollworm survival during July in the adult vial test at 5 µg cypermethrin/vial during 2008-2010						
compared to 1998-2000.						
	% Survival (Number tested)					
State	1998-2000	2008-2010	Change			

	% Survival (Number tested)			
State	1998-2000	2008-2010	Change	
Arkansas	3.8	18.7	+14.9	
Georgia	9.6	15.4	+5.8	
Louisiana	11.7	47.0	+35.3	
Mississippi	0.0	12.6	+12.6	
Missouri	12.4	8.4	-4.0	
South Carolina	10.9	9.9	-1.0	
Tennessee	0.0	14.6	+14.6	
Texas	4.5	6.3	+1.8	
Virginia	9.5	31.2	+21.7	
Overall	8.2	18.2	+10.0	

### **Conclusions**

Bollworm resistance to pyrethroid insecticides has increased across the cotton belt during the last decade. The highest survival rates were found in Louisiana each year. Survival rates were highest during July. Carbon isotope testing indicates that most moths during July came from  $C_4$  plants, which corresponds to the time when bollworm moths would be emerging from corn fields. There were no differences between resistant and susceptible moths in the proportion coming from  $C_4$  plants, so there is no evidence that pyrethroid resistance is due to selection in any particular crop, but is likely due to the cumulative effects of minor selection in many crops.

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