PYRETHROID RESISTANCE MONITORING OF BOLLWORMS

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Abstract

Bollworms develop on many hosts, with each generation likely to use a different host. Pyrethroid insecticides are typically used to control bollworms in cotton as well as in numerous other crops that are attacked by this insect. Therefore, selection for pyrethroid resistance within the landscape is potentially great even though there may not be much selection pressure in any single environment. Pheromone traps were monitored from May to September during 2007, 2008 and 2009 to collect male moths that were then tested for resistance to a pyrethroid insecticide. Average survival at 5 µg/vial of cypermethrin over the three years was 16.3%, with Louisiana, Texas and Virginia exceeding 20% average survival. Average survival was higher in all states when compared to 1998 data. Survival during July and August is higher than survival in May or September. Carbon isotope analysis indicates that most of these mid-summer moths developed as larvae on grasses, most likely corn and sorghum. Monitoring will continue in the future to document the impact of newer transgenic corn varieties that are more toxic to bollworms.

Introduction

Bollworm, *Helicoverpa zea*, is a pest in numerous agronomic crops where it can be subjected to selection by applications of pyrethroid insecticides. It has a short generation time and is highly mobile, so large populations can build quickly and then disperse. Although transgenic Bt-cottons provide some control of this pest, it is still the second most damaging pest in cotton (Williams 2009). A pyrethroid insecticide application has been a standard control tactic used for many years. Populations of a closely related species, tobacco budworm, *Heliothis virescens*, have been resistant to pyrethroids since at least the 1980s (Luttrell et al. 1991). Given pyrethroid selection in numerous crops, it is possible that pyrethroid resistance will develop in bollworm, and once it develops it will likely become widespread. Pyrethroids are still the primary foliar chemistry used against bollworms, so knowledge of resistance and how it is developing and spreading is critical to avoid ineffective insecticide applications and yield losses in cotton.

Monitoring pyrethroid resistance in bollworms has been conducted for numerous years using an adult vial test. 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 dose of 5 µg 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 µg/vial dose (Leonard, B.R., unpublished data). Pyrethroid monitoring is also occurring in sweet corn growing regions, with increasing survival in the Mid-Atlantic states (Payne et al. 2006).

Data in both Louisiana and the Mid-Atlantic states indicate that pyrethroid susceptibility varies over the course of the year. It is hypothesized that this is a result of variable selection pressure in moths coming from different crops and/or different regions. A recently developed tool allows us to determine the host plant of the larva from examining the adult. Bollworm moths have been shown to retain in their wings a very similar ratio of ^{13}C:^{12}C as their natal host plant (Gould et al. 2002). Terrestrial plants generally utilize one of two photosynthetic pathways: C_3 or C_4. Plants utilizing the C_3 photosynthetic pathway are more depleted in ^{13}C compared to C_4 plants. Thus, the ratio of ^{13}C:^{12}C differs between plants using the C_3 and C_4 photosynthetic pathways, as well as insects that develop on these plants. By using this tool, we can determine if the larva developed on a broadleaf (mostly C_3) or a grass (C_4) such as corn. Combined with knowing the densities of crops growing in a region and when those crops are producing *H. zea* adults (Jackson et al. 2008), larval host information can help identify likely source regions for the current generation.

This project monitored pyrethroid resistance from numerous locations in the cotton belt throughout the season. Furthermore, resistant moths and a subsample of susceptible moths were retained for further testing of carbon isotopes. Resistance data reported are from 2007-2009. Carbon isotope analysis is has been completed for 2007 moths and is partially completed for 2008 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 the bollworm. 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 µg 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 µg 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 is 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 (‰) were considered to have a C_4 host signature, whereas moths with $\delta^{13}C$ values between -28 and -20‰ 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 3908 moths collected in 2007 and 1877 moths from 2008. Additional 2008 moths and all moths from 2009 remain to be analyzed.

Results and Discussion

Over all sites in the 9 participating states throughout 2007-2009, 16.3% of more than 34,000 tested moths survived a concentration of 5 µg cypermethrin / vial. Within the three years, there was no obvious trend overall, but the survival rates of individual states varied widely (Table 1). Louisiana had the highest survival rate over the three years. Annual trends show substantial shifts in survival for some states. Virginia has had a large increase in survival from 2007 to 2009 while Texas, Georgia and South Carolina all recorded large decreases in survival. Tennessee had high survival in 2007 but had values similar to neighboring states in 2008 and 2009. At this point these annual variations cannot be explained, but given the quantity of moths tested in these states, they are believed to reflect changes in the tested populations. While overall increasing resistance was not observed within the three years of testing, survival rates at 5 µg cypermethrin / vial increased in all 7 states monitored both in this study and in studies conducted in 1998 and 1999 (Martin et al. 1999, 2000). Average survival in 1998-1999 was 5.8%, rising to 16.2% during 2007-2009 (Table 2). An analysis of monthly trends in all states shows a similar pattern of increased survival from May to July followed by a plateau or slight decrease in survival (Fig. 1). Increased survival during mid-summer observed here is in contrast to 1998 and 1999 data that showed the opposite trend of lower survival in mid-summer (Martin et al. 1999, 2000).

Table 1. Annual bollworm average survival in the adult vial test at 5 µg cypermethrin/vial.

	% Survival (Number tested)		
State	2007	2008	2009
Arkansas	5.5 (123)	16.0 (2443)	15.0 (1285)
Georgia	28.9 (958)	12.2 (1252)	8.6 (847)
Louisiana	29.3 (678)	33.3 (1062)	40.1 (856)
Mississippi	9.8 (2890)	7.8 (2504)	11.5 (3661)
Missouri	16.7 (1644)	11.2 (1382)	8.7 (1480)
S. Carolina	23.8 (705)	6.0 (415)	7.0 (553)
Tennessee	37.5 (297)	14.8 (503)	14.5 (822)
Texas	39.0 (1204)	14.8 (1070)	4.4 (821)
Virginia	4.5 (765)	19.6 (1597)	24.7 (2395)
Overall	19.4 (9264)	15.0 (12,228)	15.3 (12,720)

Table 2. Average bollworm survival in the adult vial test at 5 µg cypermethrin/vial during 2007-2009 compared to 1998-1999.

	% Survival (Number tested)		
State	1998-1999	2007-2009	Change
Arkansas	6.4 (392)	15.6 (3851)	+9.2
Georgia	2.1 (1266)	16.4 (3057)	+14.3
Louisiana	12.6 (1413)	34.6 (2596)	+22.0
Mississippi	0.2 (835)	9.7 (9055)	+9.5
Missouri	8.0 (276)	12.2 (4506)	+4.2
South Carolina	4.6 (2796)	13.6 (1673)	+9.0
Tennessee	6.0 (532)	18.7 (1622)	+12.7
Texas	2.1 (819)	22.5 (3095)	+20.4
Overall[1]	5.8 (10,345)	16.3 (34,212)	+10.5

[1]Overall data includes data from states not shown because they were not tested in both time periods.

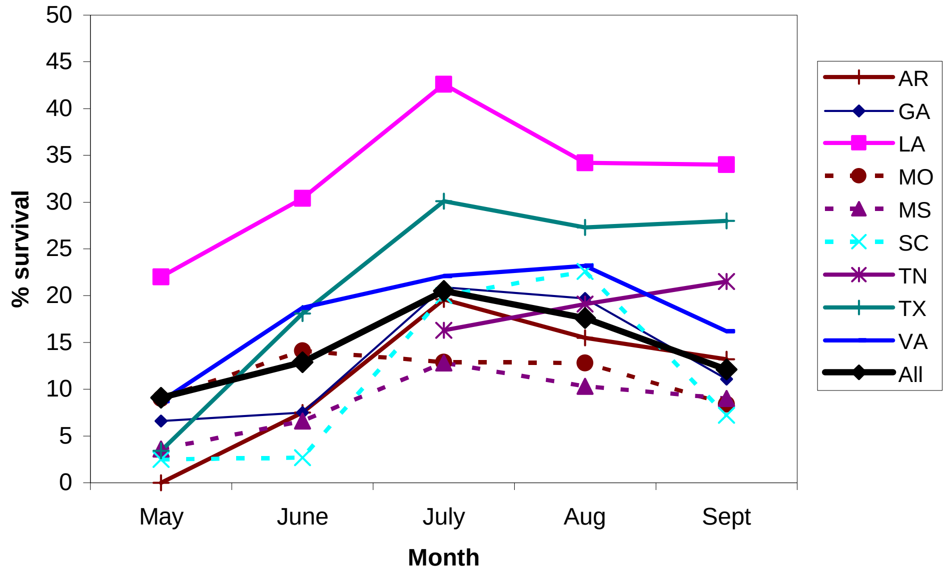

Figure 1. Average bollworm survival in the adult vial test at by month at 5 µg cypermethrin/vial during 2007-2009.

The 2007 carbon isotope data shows that the highest moth survival in July occurred when nearly all of the moths developed on C_4 hosts (Fig. 2). The 2008 data look similar but are not presented as this analysis is incomplete. The landscape across the cotton belt has changed dramatically over this 10-year period with corn being a much more dominant crop now and cotton being less common. Because bollworms developed in corn are more fit than other bollworms (R.E. Jackson, unpublished data), this higher mid-summer survival is likely a function of more fit moths being tested. Furthermore, the carbon isotope data do not indicate substantial pyrethroid resistance selection as the proportions of resistant and susceptible moths coming from C4 plants are similar throughout the growing season (Fig. 3). Based on the pyrethroid resistance and carbon isotope data presented here, cotton does not appear to be a key host in bollworm population dynamics or pyrethroid selection at the current time. With the widespread use of Bt cotton varieties, this is not surprising. Widespread planting of newer Bt corn varieties that are more toxic to bollworms may reduce the role of corn and cotton may again become a more substantial factor in bollworm population dynamics. The impact of these changes on pyrethroid resistance is unknown.

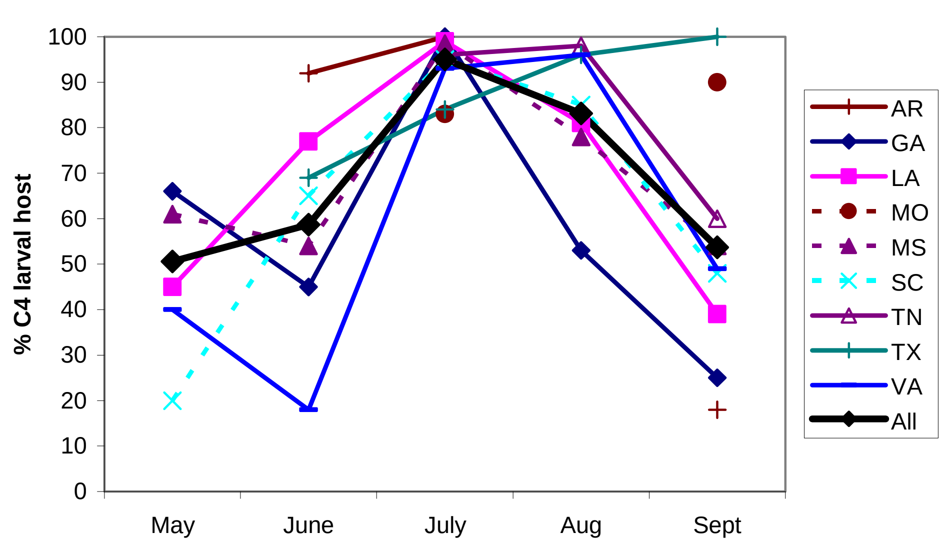

Figure 2. Percent C_4 larval host plants in each state by month during 2007.

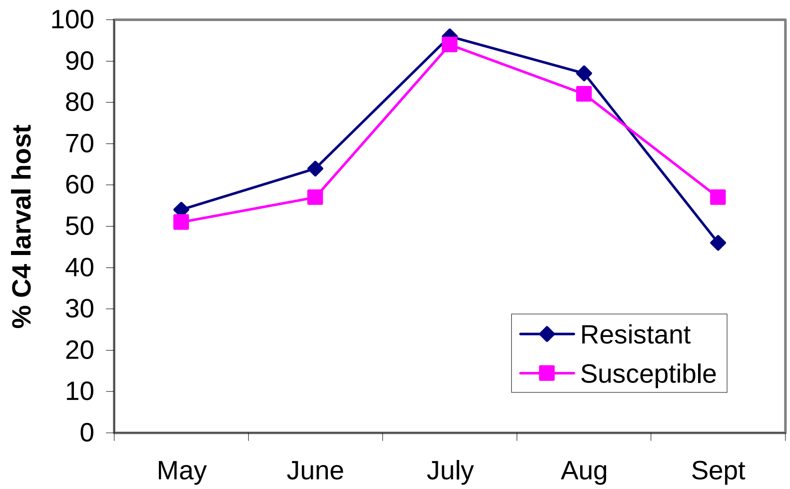

Figure 3. Percent C_4 larval host plants for moths surviving the 5 µg cypermethrin/vial concentration (resistant) compared to those dying at this concentration. Average of all states shown.

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. Virginia had high levels of resistance during 2008 and 2009. Survival rates were highest during July and August. Carbon isotope testing indicates that most moths during July and August come 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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