INSECTICDE RESISTANCE MONITORING UPDATE: BOLLWORMS, LOOPERS AND TARNISHED PLANT BUGS

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Abstract

Soybean looper populations from Virginia, South Carolina and Mississippi were screened for resistance to methoxyfenozide, spinosad, and chlorantraniliprole. Compared to a laboratory colony, no populations were resistant to any insecticides. Nineteen to 20 tarnished plant bug populations from various parts of Mississippi were screened for resistance to three commonly used insecticides. Susceptibility to all insecticides was highly variable with up to 10, 32 and 37% of the populations tested considered potentially resistant to imidacloprid, sulfoxaflor and thiamethoxam, respectively. Tarnished plant bugs collected from the Delta and Northeastern Hills regions of Mississippi were similar in their likelihood to be resistant to the insecticides. Bollworm research focused on developing a rapid screening protocol that could be used to monitor for diamide resistance. Studies were conducted to evaluate the impact of environmental conditions (temperature, UV light and time) on the durability of insecticide residues on vials. Results suggested that diamide-treated vials were stable for at least 3 months and shipping and storage conditions had little impact on performance. Treated vials were tested in Arkansas, Mississippi and South Carolina on wild bollworm moths caught in pheromone traps. Insecticide efficacy was variable by location and time of year. In 2017, the moths in South Carolina survived the best, while in 2018 the moths in Arkansas survived the best. It is unknown if the variability detected in this assay reflects a genetic difference in diamide susceptibility, or if it is responding to other differences in moths.

Introduction

Insecticide resistance is an ongoing problem for management of numerous insects. Routine monitoring can provide an early warning system before field failures become common, alerting growers when they can no longer assume that an insecticide will provide good control. It is also helpful to remind growers that resistance is always developing at some rate, and that they need to manage their use of insecticides if they want them to be effective for a long time.

Insecticide resistance monitoring can be done in multiple ways. A common method for Lepidoptera is to collect larvae from a field, rear the insects in the laboratory until the next generation of larvae have emerged and then assay small larvae of uniform age with an insecticide (Adamczyk et al. 1999, Temple et al. 2009). While effective, this method is time consuming and doesn't provide any data for at least one generation, which is often about a month after collection, so it does not provide data in a timely manner for making management decisions. A second approach that is used with Lepidoptera is to collect moths in a pheromone trap and assay the moths (Pietrantonio et al. 2004, Musser et al. 2010, van Kretschmar et al. 2013). This has the advantage of providing results within a day and not requiring any insect rearing, but the relationship between adult susceptibility and larval susceptibility is often unknown and may not be consistent. When the adult is a pest stage as with tarnished plant bug, adults can be collected and tested (Xu and Brindley 1993, Shelton et al. 2006, Snodgrass et al. 2008). A limitation in this situation is that populations may appear more or less resistant based on the quality of the habitat from where they were collected rather than due to genetic variability.

This manuscript covers results from larval assays on soybean looper, adult assays on tarnished plant bug, and adult assays on bollworm. These efforts are ongoing, so this should be considered a progress report rather than a final statement.

Materials and Methods

Soybean Looper Assays

Populations of soybean looper were collected in Mississippi, North Carolina, South Carolina, and Virginia during July and August, 2018. These four populations plus a laboratory colony were assayed to estimate their susceptibility to chlorantraniliprole, methoxyfenozide, and spinosad. Field populations were assayed in the F_1 and/or F_2 generations. All larvae were collected from unsprayed soybean fields, brought to the Mississippi State University Insect Rearing

facility in Starkville, MS, and reared on standard artificial diet. A diet-incorporation assay was conducted on 3rd instar larvae weighing 20-40 mg as described by Owen et al. (2013). For each insecticide, five concentrations of active ingredient plus a control were created using serial dilutions to span the concentrations expected to provide partial mortality. Formulated insecticides were used for all assays, namely Prevathon 0.43 SC, Blackhawk, and Intrepid 2F were used for chlorantraniliprole, spinosad, and methoxyfenozide assays, respectively. A minimum of 17 larvae per concentration per replicate were tested, depending on the availability of larvae. Replicates were assays conducted on different days from the same generation of eggs. Mortality was determined after 96 hr. Because chlorantraniliprole primarily kills by preventing larvae from feeding, the determination of mortality for chlorantraniliprole was larvae that weighed less than 50 mg. Most larvae in the controls weighed more than 150 mg and none were under 100 mg, so any larvae failing to exceed 50 mg were severely stunted by the insecticide, and when some were kept longer to observe their eventual fate, they never matured. Insects were considered dead in spinosad and methoxyfenozide assays if they could not right themselves when disturbed. LC_{50} and LC_{90} estimates were made using Proc Probit in SAS 9.4 (SAS Insitute Inc., Cary, NC). Estimates were corrected for control mortality (Abbott 1925). When an individual replicate failed to generate a significant slope ($P>0.05$) in probit analysis, that replicate was removed from the data set. This occurred for three of four North Carolina assay replicates plus six other assay replicates out of 52 total that were conducted.

Tarnished Plant Bug Assays

Adult tarnished plant bug populations were collected from uncultivated flowering plants (e.g. daisy fleabane) throughout Mississippi cotton growing regions during May-August, 2017 and 2018 using sweep nets. Insects were aspirated into containers and fed green beans and artificial diet until the assays could be conducted. Assays were conducted within 3 days of collection. Vials for sulfoxaflor assays were prepared by coating a 20 ml glass scintillation vial with 0.25 ml of various insecticide concentrations mixed in acetone. Vials were rolled under a fume hood until the acetone evaporated, leaving a uniform insecticide residue on all vial surfaces. A surface-sterilized piece of green bean was added to each vial as a food source. Thiamethoxam and imidacloprid assays were conducted by mixing various concentrations of insecticide into a 10% honey-water solution. A 12 mm diameter x 12 mm thick disk of floral foam was placed into a 20 ml glass scintillation vial and then 0.5 ml of the insecticide-honey-water solution was pipetted into the vial slowly so the solution could be absorbed by the floral foam. All assays included 5-6 concentrations with approximately 30 insects tested at each concentration. Tarnished plant bug adults were placed into the treated vials and mortality was assessed after 24 hr. Data were analyzed using Proc Probit in SAS 9.4. Assays that did not have a significant response to concentration ($P>0.05$) were deleted from the data set. To estimate baseline susceptibility of tarnished plant bug to each insecticide, the average of the six lowest LC_{50} estimates was used. Given the widespread use of these insecticides, this method likely underestimates resistance. Comparison to laboratory colonies is common, but when testing the parental population, rearing conditions can alter bioassay susceptibility, so a comparison to a laboratory colony becomes meaningless. Because poor field efficacy is not believed to be widespread for these insecticides, the lowest third LC_{50} estimates should only include populations that have not developed much resistance. Furthermore, this baseline provided a measure of variability in response, and provided a way to compare resistance levels among populations and insecticides.

Bollworm Moth Assays

The adult vial test for chlorantraniliprole was prepared by mixing formulated chlorantraniliprole (Prevathon 0.43 SC) with water and then acetone to make a stock solution. Serial dilutions with acetone were made from the stock solution to create solutions with a range of concentrations where bollworms had partial mortality. To treat a single vial, 500 µl of an insecticide solution was placed in a 20 ml scintillation vial and then the vial was rolled on a hotdog roller without a heating element inside a chemical fume hood until the acetone evaporated. This created a vial with a uniform coating of a specific diamide concentration. Vials were capped and stored at room temperature until shipped or used.

To determine the stability of vials when prepared, shipped and stored ahead of time, vials were prepared at 10 µg/vial and subjected to a variety of temperature and ultraviolet light exposure conditions potentially encountered in shipping and storage. Temperatures were tested at 0° (kept in a chest freezer), 20° (kept on a lab shelf) and 40°C (kept in a growth chamber set at 40°C). Ultraviolet (UV) light was provided by a LED grow light (Aokey 15W desk clamp plant grow light) placed directly over a tray of vials. Vials not exposed to UV light were kept in a box inside the same storage unit so that temperatures were the same for UV and no UV treatments. Conditions were maintained for 1 and 14 d and then assays with the laboratory bollworm colony were conducted using the vials to evaluate any change in efficacy. The trial was repeated at four different times (replicates) using 20 moths per treatment in each replicate. Data were analyzed using Proc Glimmix in SAS 9.4.

Based on data from the laboratory colony and 2017 data from a laboratory colony, a concentration of 10 µg/vial was used as the diagnostic concentration for the adult vial test. Vials were prepared at this concentration and shipped to Arkansas and South Carolina beginning in late May to be used over the following months along with vials treated only with acetone. Using wild male bollworm moths captured in pheromone traps, bollworms were tested at these locations and Mississippi whenever large numbers of bollworms were available. The number of moths tested varied with the number of healthy moths captured in local pheromone traps.

Results and Discussion

Soybean Looper Assays

Few larvae were shipped from North Carolina, so no assays were possible during the F_1 generation and few assays were possible in the F2 generation. The North Carolina population did not grow as well as the other populations in the laboratory, and control mortality was high in the assays. As a result, none of the assays fit a probit line well (P-value for $Ç^2$ <0.10), so no results are reported for this population. All other populations behaved similarly to the laboratory colony and had low control mortality in all assays.

No populations were more than 2.11 times more resistant to any of the tested insecticides than the laboratory colony (Tables 1, 2, and 3). Furthermore, the 95% fiducial limits of all populations overlapped, suggesting that resistance was not observed in any population to any of the insecticides tested.

Table 1. Susceptibility of soybean looper populations to chlorantraniliprole in 96-hr diet-incorporated bioassays.

Population	Generation	N[1]	LC_{50} (ppm)	RR[2]	LC_{90} (ppm)	RR[2]
MS	F_1	120	0.089 (0.056-0.134)	0.60	0.316 (0.198-0.693)	0.57
	F_2	206	0.055 (0.034-0.080)	0.38	0.341 (0.216-0.704)	0.61
SC	F_1	89	0.036 (0.004-0.086)	0.24	0.205 (0.085-1.151)	0.37
	F_2	209	0.111 (0.069-0.151)	0.75	0.289 (0.206-0.543)	0.52
VA	F_1	339	0.134 (0.008-0.277)	0.91	0.858 (0.465-3.512)	1.55
	F_2	215	0.274 (0.141-0.492)	1.87	1.414 (0.742-4.582)	2.55
Lab Colony		120	0.147 (0.095-0.223)		0.554 (0.343-1.236)	

[1] Number of larvae tested in successful assays
[2] Resistance ratio based on the estimated LC_{50} or LC_{90} of the laboratory colony

Table 2. Susceptibility of soybean looper populations to spinosad in 96-hr diet-incorporated bioassays.

Population	Generation	N[1]	LC_{50} (ppm)	RR[2]	LC_{90} (ppm)	RR[2]
MS	F_1	107	1.14 (0.92-1.40)	1.44	1.84 (1.48-2.81)	0.43
	F_2	210	1.21 (0.89-1.48)	1.52	2.51 (2.03-3.52)	0.59
SC	F_1	120	0.79 (0.27-1.40)	0.99	3.19 (1.83-6.95)	0.75
	F_2	229	0.67 (0.37-0.95)	0.84	3.33 (2.29-6.45)	0.79
VA	F_1	348	1.46 (0.08-3.46)	1.83	7.20 (2.99-72.46)	1.70
	F_2	494	1.32 (0.89-1.77)	1.66	4.45 (3.12-8.36)	1.05
Lab Colony		215	0.79 (0.31-1.34)		4.24 (2.67-8.06)	

[1]Number of larvae tested in successful assays
[2] Resistance ratio based on the estimated LC_{50} or LC_{90} of the laboratory colony

Table 3. Susceptibility of soybean looper populations to methoxyfenozide in 96-hr diet-incorporated bioassays.

Population	Generation	N[1]	LC_{50} (ppm)	RR[2]	LC_{90} (ppm)	RR[2]
MS	F_1	228	0.38 (0.12-0.73)	0.39	1.98 (1.00-10.60)	0.22
	F_2	295	0.47 (0.30-0.65)	0.48	2.63 (1.79-4.62)	0.29
SC	F_1	101	1.61 (0.75-2.53)	1.65	4.91 (3.05-15.77)	0.54
	F_2	229	1.28 (0.91-1.70)	1.30	4.29 (3.05-7.27)	0.47
VA	F_1	361	0.50 (0.24-0.84)	0.51	4.04 (2.56-7.01)	0.44
	F_2	473	2.07 (1.50-2.81)	2.11	10.53 (6.98-19.26)	1.16
Lab Colony		215	0.97 (0.00-4.73)		9.08 (1.43-12,297)	

[1]Number of larvae tested in successful assays
[2] Resistance ratio based on the estimated LC_{50} or LC_{90} of the laboratory colony

Tarnished plant Bug Assays

Susceptibility among populations varied for all three insecticides, but particularly for thiamethoxam (Table 4). Susceptibility of populations collected from the Delta region was similar to populations from the Northeastern Hills part of the state and there were no differences between 2017 and 2018. It is unknown how these levels of susceptibility correspond to field efficacy, but it is expected that the most resistant populations to thiamethoxam may not be well controlled with this chemistry.

Table 4. Susceptibility of tarnished plant bugs from Mississippi during 2017 and 2018 to imidacloprid, thiamethoxam and sulfoxaflor.

Insecticide	Region[a]	# Pop. Tested	Resistance Ratio Range[b]	# Pop. "Resistant"[c]
Admire (imidacloprid)	Delta	11	0.26 – 8.44	0 (0%)
	Hills	9	0.10 – 29.7	2 (22%)
	Overall	20	0.10 – 29.7	2 (10%)
Centric (thiamethoxam)	Delta	10	1.32 – 188.0	4 (40%)
	Hills	9	0.06 – 56.1	3 (33%)
	Overall	19	0.06 – 188.0	7 (37%)
Transform (sulfoxaflor)	Delta	9	0.62 – 18.8	3 (33%)
	Hills	10	0.70 – 37.3	3 (30%)
	Overall	19	0.62 – 37.3	6 (32%)

[a] Delta consists of the Mississippi River delta region along the western side of Mississippi. Hills consists of the cotton production areas in the northeastern part of Mississippi.
[b]Baseline estimated from the average of the lowest six LC_{50} estimates for the insecticide
[c] 10 times the average of the lowest six LC_{50} estimates for the insecticide

Bollworm Moth Assays

No tested conditions of temperature and/or ultraviolet light exposure on the vials had any impact on subsequent moth mortality (Table 5). Combined with data from 2017 that showed consistent efficacy up to 90 days after preparation (data not shown), it appears that vials can be shipped without taking any special precautions and held for up to at least 3 months without a loss of efficacy.

A total of 1623 bollworm moths were tested at 10 µg chlorantraniliprole /vial during the 2017 and 2018 growing seasons. The mortality in the three states varied, with the highest survival in South Carolina during 2017 and in Arkansas during 2018 (Fig. 1). In both locations with high survival, the assays were done on several hundred moths over several weeks and included two separate shipments of vials prepared independently, so it is believed that these assays reflect differing susceptibilities, and are not a result of any problem in methodology. Whether this level of variability observed in moths reflects differences in larval susceptibility is still unknown. While 10 µg/vial seemed like a good discriminating concentration based on the laboratory colony, perhaps a higher discriminating concentration would reduce variability and make important changes in susceptibility more apparent.

There appears to be potential for a diamide assay conducted on bollworm moths. Prepared vials appear to be stable, allowing vials to be prepared at a single lab and shipped around the country to cooperators. The discriminating concentration of 10 µg/vial may be too low for field bollworm populations, so a concentration of 15 or 20 µg/vial may be more appropriate to readily separate susceptible and resistant populations of bollworm.

Table 5. Impact of temperature and ultraviolet light on dried residues of 10 µg/vial chlorantraniliprole in vials.

Treatment	Exposure Time (days)	Temperature (° C)	UV	% Mortality (± SEM)
Acetone Control	0			8 ± 2.0b
Positive Control	0			90 ± 3.4a
Short Cold	1	0	No	85 ± 7.9a
Long Cold	14	0	No	93 ± 4.4a
Short Room	1	20	No	80 ± 7.6a
Long Room	14	20	No	82 ± 7.4a
Short Room UV	1	20	Yes	85 ± 8.9a
Long Room UV	14	20	Yes	83 ± 4.4a
Short Hot	1	40	No	86 ± 5.5a
Long Hot	14	40	No	83 ± 9.5a
Short Hot UV	1	40	Yes	89 ± 2.3a
Long Hot UV	14	40	Yes	78 ± 6.1a

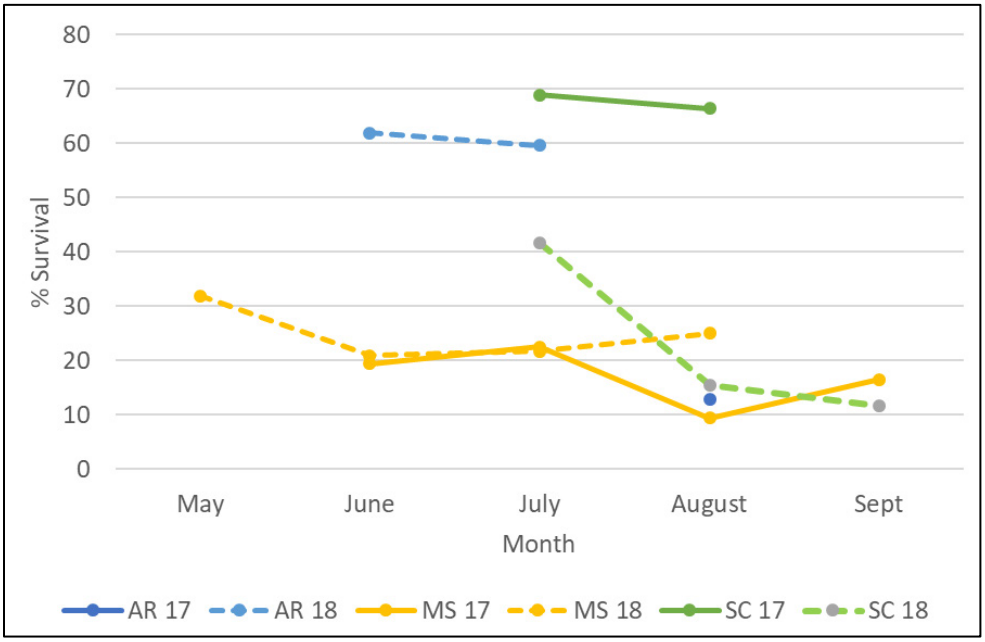

Fig. 1: Bollworm male moth mortality after 24 hr using 10 of chlorantraniliprole during 2017 and 2018. Percent survival corrected for control mortality.

Summary

Insecticide resistance was monitored with bioassays for three cotton pests. No populations of soybean looper were resistant to chlorantraniliprole, methoxyfenozide or spinosad. Tarnished plant bug susceptibility to imidacloprid, thiamethoxam and sulfoxaflor was variable, especially to thiamethoxam. Based on an unconventional baseline of susceptibility, 10-37% of the populations were determined to be resistant to the pesticides. While it is unknown if current levels of resistance result in reduced field efficacy, it is likely that previous selection has led to the large differences in susceptibility among populations, and reduced efficacy from all these pesticides is likely in the future. Bollworm moth assays with chlorantraniliprole appear to be feasible, but susceptibility of moth populations is variable. Further work is planned to establish the relationship between bollworm moth and larval susceptibility.

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