INSECTICIDE RESISTANCE MONITORING FOR BOLLWORM AND SOYBEAN LOOPER

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Abstract

Current management of Lepidoptera relies on transgenic Bt varieties and a few foliar insecticides. The development of insecticide resistance is a risk to continued efficacy of these technologies. Routine monitoring of field populations may help detect slight changes in susceptibility before control failures are observed. To document current levels of susceptibility among bollworm and soybean looper populations, annual field collections of soybean looper were made between 2018 and 2021 and during 2020 for bollworm. These collections were from the southeastern US and Puerto Rico. Populations were reared in the laboratory for one or more generations and then bioassayed with several foliar insecticides to determine an LC_{50} for each population. Susceptibility was compared to laboratory colonies. No populations of bollworms were resistant to chlorantraniliprole or indoxacarb. No populations of soybean looper were resistant to spinosad, methoxyfenozide, chlorantraniliprole, or indoxacarb except one collection from Puerto Rico, which was resistant to spinosad, methoxyfenozide, and chlorantraniliprole. Further monitoring is planned along with more genetic analysis of resistance mechanisms.

Introduction

Bollworm, *Helicoverpa zea* (Boddie), and soybean looper, *Chrysodeixis includens* (Walker), are economically important pests of cotton and other agronomic crops grown in the southern US. Management of these pests includes planting transgenic Bt varieties and applying foliar insecticides. Resistance to several insecticides has been documented among these species (Felland et al. 1990, Leonard et al. 1990, Mink and Boethel 1992, Mascarenhas and Boethel 1997, Stadelbacher et al. 1990, Sparks 1981, Jacobson et al. 2009, Tabashnik and Carrière 2010), so maintaining susceptibility to currently used insecticides is a priority. Routine monitoring of susceptibility can provide an early warning system of changes in susceptibility before field failures are observed, alerting growers and consultants when they should no longer assume that an insecticide will provide good control, and encouraging good stewardship of these pesticides.

A common method of monitoring insecticide resistance in 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. However, it may be useful for detecting small shifts in susceptibility before field control failures occur. Therefore, insecticide bioassays were conducted on field collections of bollworm and soybean looper between 2018 and 2021.

Materials and Methods

Bollworm Collections

Populations of bollworms were collected during June-July 2020 from multiple southeastern US states (Table 1). Collections were made from corn, grain sorghum and clover. In addition, a long-term laboratory colony was reared on artificial diet at the Mississippi State University Insect Rearing Center. Third instar larvae (20-40 mg) of both field collections and the laboratory colony were assayed on five concentrations plus a control of chlorantraniliprole and indoxacarb using the diet-incorporation method (Owen et al. 2013). Field populations were assayed during the F1 and/or F2 generations.

Soybean Looper Collections

Populations of soybean looper were collected during July-September 2018-2021 from multiple southeastern US states and Puerto Rico. Several collections were lost before any assays could be conducted. Those collections from which data were collected are listed in Table 1. Collections were made from untreated soybean fields. In addition, a long-term laboratory colony was reared on artificial diet at the Mississippi State University Insect Rearing Center. Third instar larvae (20-40 mg) of both field collections and the laboratory colony were assayed on five

concentrations plus a control of chlorantraniliprole, methoxyfenozide, spinosad, and indoxacarb using the diet-incorporation method (Owen et al. 2013). Field populations were assayed as soon as possible, but due to the number of chemicals being tested and challenges in getting some field populations established, most assays were conducted during the F_2 and F_3 generations.

Table 1. Collection locations with bioassay data for bollworm and soybean loopers

Species	Year	Locations
Bollworm	2020	Arkansas, Georgia, Louisiana, Mississippi (2), North Carolina (2), South Carolina, Tennessee, Virginia
Soybean Looper	2018	Mississippi, South Carolina, Virginia
	2019	Arkansas, Mississippi, North Carolina, South Carolina, Tennessee, Virginia, Puerto Rico
	2020	Mississippi
	2021	Louisiana, Mississippi, South Carolina

Insecticide Bioassays

Assays were replicated on separate days with a minimum of 20 larvae used per concentration per day. Mortality was determined after 96 hr. Because chlorantraniliprole primarily kills by preventing larvae from feeding, the determination of mortality for all assays was larvae that weighed less than 50 mg or could not right themselves when disturbed. 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. LC50 estimates were made using Proc Probit in SAS 9.4 (SAS Institute 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. Resistance ratios were calculated based on the LC_{50} of the field collection divided by the LC_{50} of the laboratory colony during the same year. A field collection was not considered resistant to an insecticide unless the resistance ratio was >8.

Results and Discussion

Ten bollworm populations were tested for susceptibility to chlorantraniliprole during 2020. All populations were similar to the laboratory colony with no population having a resistance ratio greater than 1.8 (Table 2). Similarly, nine of the collections were tested for susceptibility to indoxacarb and all had a resistance ratio of 1.5 or less (Table 2), so there was no indication that there was any resistance to either insecticide among the bollworm collections tested.

A total of 14 field collections of soybean looper were tested for susceptibility to chlorantraniliprole between 2018 and 2021. While susceptibility to chlorantraniliprole was more variable among soybean looper collections than among bollworm collections, the only collection considered resistant to chlorantraniliprole was the 2019 collection from Puerto Rico (Table 3). The results for susceptibility to spinosad and methoxyfenozide were similar in that the only collection considered resistant to these insecticides was the 2019 collection from Puerto Rico (Tables 3 and 4). No resistance to indoxacarb was documented for the two collections tested (Table 4).

Only one replication of assays could be completed on the 2019 Puerto Rico collection because the colony crashed in the laboratory. Additional collections were made from Puerto Rico during 2020 and 2021, but the populations could not be established to conduct any assays. It is unknown whether the difficulty of getting the Puerto Rico collections established is related to insecticide resistance or not. Further efforts are underway to establish a collection of soybean looper from Puerto Rico and to analyze the resistance mechanisms found in the population.

Table 2. Bollworm susceptibility to chlorantraniliprole and indoxacarb during 2020

	Chlorantraniliprole			Indoxacarb		
Population	**Reps**	**LC_{50} (ppm)**	**Resistance Ratio**	**Reps**	**LC50 (ppm)**	**Resistance Ratio**
Lab	3	0.015 (0.012-0.017)	1	4	0.10 (0.089-0.13)	1
AR	2	0.025 (0.021-0.030)	1.7	4	0.12 (0.11-0.12)	1.2
GA	2	0.023 (0.016-0.031)	1.5	-	-	
LA	3	0.027 (0.018-0.035)	1.8	4	0.11 (0.078-0.13)	1.1
MS-1	3	0.021 (0.016-0.028)	1.4	2	0.15 (0.14-0.16)	1.5
MS-2	2	0.019 (0.014-0.025)	1.3	3	0.11 (0.090-0.13)	1.1
NC-1	3	0.014 (0.009-0.018)	0.9	3	0.10 (0.058-0.12)	1.0
NC-2	3	0.021 (0.017-0.027)	1.4	2	0.12 (0.10-0.13)	1.2
SC	3	0.020 (0.017-0.023)	1.3	4	0.11 (0.088-0.14)	1.1
TN	3	0.023 (0.016-0.028)	1.5	1	0.13 (0.11-0.16)	1.3
VA	2	0.020 (0.012-0.026)	1.3	3	0.12 (0.10-0.14)	1.2

Table 3. Soybean looper susceptibility to chlorantraniliprole and spinosad

		Chlorantraniliprole			Spinosad		
Population	**Year**	**Reps**	**LC_{50} (ppm)**	**Resistance Ratio**	**Reps**	**LC50 (ppm)**	**Resistance Ratio**
Lab	2018	2	0.140 (0.023-0.264)	1	2	0.79 (0.31-1.34)	1
Lab	2019	2	0.249 (0.053-0.492)	1	2	0.20 (0.016-0.48)	1
Lab	2020	3	0.180 (0.078-0.290)	1	2	0.95 (0.15-1.66)	1
Lab	2021	1	0.227 (0.150-0.309)	1	3	0.69 (0.55-0.82)	1
MS	2018	4	0.083 (0.055-0.12)	0.6	3	1.25 (1.01-1.45)	1.6
SC	2018	4	0.10 (0.046-0.16)	0.7	3	0.77 (0.55-0.98)	1.0
VA	2018	5	0.20 (0.039-0.35)	1.4	8	1.41 (1.04-1.80)	1.8
AR	2019	1	0.29 (0.14-0.53)	1.1	-	-	-
MS	2019	2	0.24 (0.06-0.54)	1.0	1	1.13 (0.38-1.96)	5.6
NC	2019	2	0.10 (0.066-0.15)	0.4	3	0.54 (0.027-1.04)	2.6
SC	2019	1	0.51 (0.011-0.85)	2.0	1	0.55 (0.052-1.18)	2.7
TN	2019	1	0.72 (0.18-1.26)	2.9	2	0.73 (0.16-1.66)	3.6
VA	2019	1	0.16 (0.12-0.21)	0.6	1	0.52 (0.001-1.02)	2.6
PR	2019	1	>2	>8	1	>8	>39.4
MS	2020	4	0.15 (0.10-0.20)	0.8	2	0.86 (0.55-1.16)	0.9
LA	2021	2	0.23 (0.17-0.31)	1.0	-	-	-
MS	2021	3	0.16 (0.096-0.23)	0.7	3	0.56 (0.017-1.15)	0.8
SC	2021	1	0.19 (0.067-0.36)	0.8	-	-	-

Summary

While no bollworm or soybean looper populations collected in the continental U.S. were resistant to any of the insecticides tested, a soybean looper collection from Puerto Rico was resistant to chlorantraniliprole, methoxyfenozide and spinosad. Given the migratory behavior of these moths, insecticide resistant populations in Puerto Rico could lead to resistant populations in the U.S. mainland in the future, so continued monitoring and vigilance is necessary.

Table 4. Soybean looper susceptibility to methoxyfenozide and indoxacarb

			Methoxyfenozide			Indoxacarb	
Population	**Year**	**Reps**	**LC_{50} (ppm)**	**Resistance Ratio**	**Reps**	**LC50 (ppm)**	**Resistance Ratio**
Lab	2018	1	0.40 (0.085-0.940	1	-	-	-
Lab	2019	2	0.93 (0.56-1.40)	1	-	-	-
Lab	2020	2	0.44 (0.054-1.53)	1	3	0.39 (0.23-0.51)	1
Lab	2021	3	0.57 (0.25-0.98)	1	3	0.48 (0.43-0.78)	1
MS	2018	5	0.42 (0.28-0.58)	1.1	-	-	-
SC	2018	3	1.36 (1.03-1.74)	3.4	-	-	-
VA	2018	7	1.18 (0.84-1.62)	2.9	-	-	-
AR	2019	1	0.71 (0.15-1.32)	0.8	-	-	-
MS	2019	2	0.43 (0.006-1.41)	0.5	-	-	-
NC	2019	3	0.43 (0.001-3.05)	0.5	-	-	-
TN	2019	2	0.94 (0.001-5.54)	1.0	-	-	-
PR	2019	1	>20	>21.6	-	-	-
MS	2020	1	2.08 (1.50-2.72)	4.7	2	0.59 (0.45-0.78)	1.5
LA	2021	2	2.15 (1.51-2.95)	3.8	-	-	-
MS	2021	3	0.77 (0.32-1.42)	1.4	4	0.82 (0.59-1.37)	1.7

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