Title: Development of Molecular Diagnostic Method for the Diamide Resistance in Soybean Looper. Project # 32-2021

2021-2022 MSPB Annual Report

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Background and Objectives

The soybean looper (*Chrysodeixis includens*) is one of the most serious pests in soybeans, migrating from southern latitudes up through Mississippi and consuming massive amounts of foliage in soybean. Since the introduction of the diamide insecticides such as chlorantraniliprole, the soybean looper has been well managed without any resistance issues. Diamides belong to a class of insecticides that target ryanodine receptors (RyR), the intracellular calcium channels that play an important role in muscle and nerve functions. However, soybean loopers have a long history of resistance to other insecticides such as pyrethroids, carbamates, and organophosphates. Therefore, diamide resistance in soybean looper is expected to develop, and growers should be prepared to manage loopers without diamides in the future. Resistance to diamides has already been reported in some lepidopteran pests from different regions in the world. Although diamide resistance hasn't been reported in soybean loopers, it seems that reduced diamide efficacy has been observed in the southeastern USA, probably those migrating from Puerto Rico. Therefore, it is important to regularly monitor populations in Mississippi in order to help farmers decide what kind of insecticide chemistries will be most efficacious. Although it is more directly reflecting the field situation, the bioassay method takes time and efforts to confirm the resistance status, because it is required to collect field populations alive and rear them in the controlled lab for a couple of generations before the bioassay. However, if there are any molecular markers to distinguish the resistant from the susceptible strain, it would be faster and easier to detect resistance, because it is not required to rear insects for testing, but it only needs individual samples. Also, the molecular method doesn't require the live caterpillars, instead any other developmental stages of the insect like adults or pupa, or any part of the body like legs can be used to detect the resistance marker, because the resistance marker has been imprinted in DNA of the pest species. Therefore, **the goal of this project is to develop a rapid and accurate diagnostic method based on DNA sequences to screen populations of soybean looper for resistance to diamide insecticides.** To achieve this goal, we postulate four objectives for year 1 with a fifth overall project objective that will be initiated in years 2 and 3.

1. Establish and maintain a resistant strain of soybean looper from Puerto Rico
2. Make comparisons between susceptible and resistant strains of the diamide resistance gene
3. Conduct standard diamide bioassays for soybean looper populations collected in Mississippi
4. Develop a molecular diagnostic marker to detect resistant soybean looper larvae
5. Monitor resistance in field populations with a molecular diagnosis kit

Impacts and Benefits to Mississippi Soybean Producers

We expect to develop a rapid diagnostic kit which can be used to screen a wide range of soybean fields in Mississippi and beyond. Results will be available to growers within a day or two of collecting the sample so that management decisions can be made with knowledge of whether a population is resistant to diamides. This new method, once confirmed, can be used by local extension agents that are equipped with minimal training and equipment.

End Products–Completed or Forthcoming

Oral presentation

Isbilir, S., Musser, F. R, Ahn, S.-J. 2021. Molecular cloning of ryanodine receptor, a target of diamide insecticides, in soybean looper, *Chrysodeixis includens*. The 85th Annual Mississippi Academy of Sciences Meeting, August 5-6, Biloxi, MS.

Isbilir, S., Musser, F. R., Ahn, S.-J. 2021. Molecular cloning of a ryanodine receptor, a target of diamide insecticides, in the soybean looper, *Chrysodeixis includens*. The 10th Annual Meeting of MEA and MAPPN, November 8-9. Mississippi State, MS.

Isbilir, S., Catchot, B., Musser, F. R., Ahn, S.-J., 2022. Expression profiles of the ryanodine receptor, a target of diamide insecticides, in soybean looper, *Chrysodeixis includens*. The 86th Annual Mississippi Academy of Sciences Meeting, March 31-April 1, Biloxi, MS.

Poster presentation

Isbilir, S., Catchot, B., Musser, F. R., Ahn, S.-J. 2021. Molecular cloning of a ryanodine receptor, a target of diamide insecticides, in the soybean looper, *Chrysodeixis includens*. BCH-EPP Student Research Symposium, Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, November 19. Mississippi State, MS.

Isbilir, S., Catchot, B., Musser, F. R., Ahn, S.-J., 2022. Expression profiles of the ryanodine receptor, a target of diamide insecticides, in soybean looper, *Chrysodeixis includens*. 2022 Joint SEB & APS-CD Meeting, March 26-30. San Juan, Puerto Rico (Virtual)

Publication *(Forthcoming)*

Isbilir, S., Catchot, B., Musser, F. R., Ahn, S.-J. *(in preparation)*. Molecular cloning and expression pattern of a ryanodine receptor in the soybean looper moth, *Chrysodeixis includens*.

Graphics/Tables

Table 1. Susceptibility of soybean looper populations to chlorantraniliprole in diet-incorporated bioassays 96 hr after exposure. Resistance ratios (RR) are compared to the laboratory colony.

Population	Gen.	N[1]	LC_{50} (ppm)	RR
Mississippi-1	F_2	241	0.173 (0.099-0.257)	0.8
Mississippi-1	F_3	120	0.117 (0.040-0.192)	0.5
Louisiana	F_3	240	0.233 (0.165-0.306)	1.0
South Carolina	F_3	121	0.188 (0.067-0.364)	0.8
Lab		174	0.227 (0.150-0.309)	

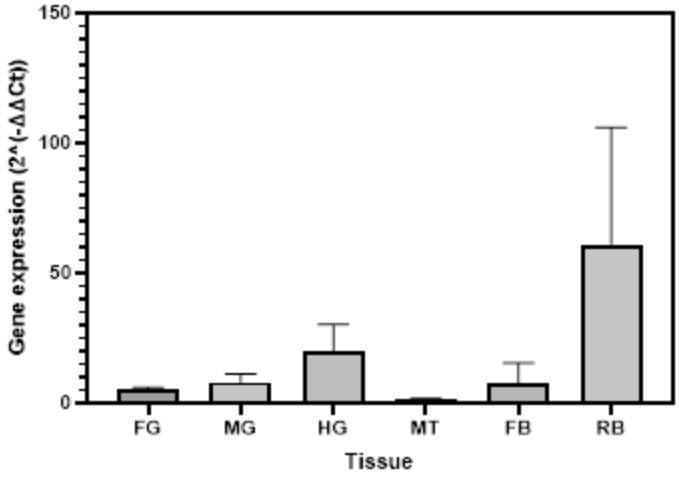

Figure 1. Quantitative real time PCR (qRT-PCR) analysis of CincRyR gene expression in 6 different tissues. (FG, forgut; MG, midgut; HG, hindgut; MT, Malpighian tubules; FB, fat body; RB, rest body)

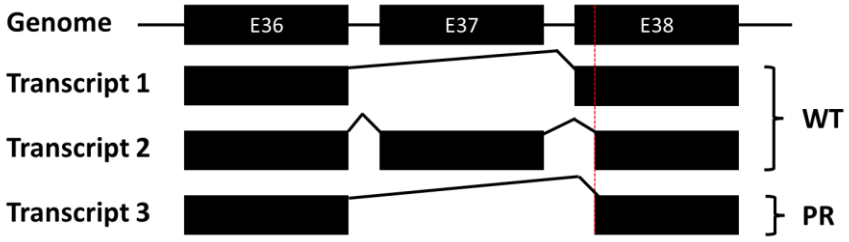

Figure 2. Alternative splicing variants of a fragment of RyR gene in soybean looper. The locus spanning exons E37-E38 shows variable transcripts which might be a candidate of resistance-associated markers.