Title: Extension and research to combat insecticide-resistant soybean aphids

Final Report for YEAR 2 (October 2019)

OBJECTIVE 1. Landscape assessment of pyrethroid resistance in Iowa (O'Neal and Hodgson)
Soybean aphid outbreaks in Iowa are erratic and difficult to predict, but occur most frequently in northern counties. More consistent and intense outbreaks occur in southern Minnesota, and this difference likely contributed to pyrethroid resistance occurring first in Minnesota. Given the confirmation of the aphid 17 years ago, it is highly likely that pyrethroid-resistant populations will spread throughout Iowa if a mitigation plan is not developed and implemented.

Objective 1a. Bioassays for pyrethroid resistance. We will document the degree and extent of pyrethroid resistance in Iowa. Since the area of Iowa potentially impacted by soybean aphid is large, it will be a challenge to locate fields infested with pyrethroid-resistant aphids. Thus, we will work with ISU Field Agronomists, CCAs, ISA On-Farm Network, and farmers to identify fields with performance issues. Aphids will be collected from these fields and screened per the methods used previously to confirm the occurrence of resistance (Hanson et al. 2017). This non-random approach in Years 1-3 will help us establish locations where this problem occurs. In Years 2 and 3, we will conduct a survey in which we randomly select fields. This approach will allow us to provide a more objective measure of the risk of insecticide-resistant aphids to Iowa farmers.

UPDATE: We collected aphids in three locations in Iowa and one in Minnesota this summer. Aphids were screened for insecticide resistance using a glass-vial bioassay treated with lambda-cyhalothrin and bifenthrin.

What it means for farmers: Insecticide resistant aphids are found in Iowa, and have been found every year since 2016. The amount of these aphids within a population is high enough to suggest that these traits are fixed and will not go away when insecticides are not used.

Objective 1b. Maintain soybean aphid colonies. A colony of pyrethroid-resistant aphids will allow more efficient laboratory research outlined for Objectives 2 and 3. This colony will be established in 2018 and maintained during the lifetime of this project. Research objectives beyond this proposal could be developed from these resistant aphid colonies (e.g., fitness costs associated with insecticide resistance).

UPDATE: Four pyrethroid-resistant colonies are being maintained in our laboratory since 2018. In addition, aphids collected this summer from the same locations specified on objective 1a are being kept in mini-colonies for further studies.

What it means for farmers: The Soybean Entomology Laboratory maintains a resource that is a value to agribusiness and scientist exploring the threat of insecticide resistance in the soybean aphid. We will continue to explore these colonies to determine the mechanism and means to manage soybean aphids as pyrethroid resistance spreads. We have shared these resources with agribusiness when requested.

<u>OBJECTIVE 2. Understanding the mechanism of resistance</u> (Coats)

One key aspect of addressing insecticide resistance is understanding how it happens. The mechanism may be based on adaptation of the population 1) at the target-site (nerve sodium channel, in the case of pyrethroids); 2) through slower penetration of the insecticide through the cuticle; 3) through rapid elimination of the insecticide; 4) through rapid detoxification of the insecticide; or 5) through aversion to the treated area (behavioral resistance). Resistance that is due to multiple mechanisms can also develop. The comparative studies with resistant and susceptible colonies will be crucial to studying the mechanism of resistance for soybean aphid.

Objective 2a. Screening aphid susceptibility to alternative insecticides. Two laboratory colonies (resistant and susceptible) will be compared for susceptibility to pyrethroids for which field failures have occurred, ʎ-cyhalothrin and bifenthrin. Other pyrethroids will also be screened for toxicity in laboratory topical-application bioassays: permethrin, esfenvalerate, cypermethrin, and deltamethrin. Commonly-used insecticides from other classes will also be tested on the two aphid colonies: neonicotinoids (imidacloprid), organophosphate (chlorpyrifos), and others.

UPDATE: Leaf dip bioassays were performed using the soybean aphid colony most resistant to lambda-cyhalothrin and a susceptible population to lambda-cyhalothrin. The two colonies were compared for susceptibility to bifenthrin, flonicamid, flupyradifurone, sulfoxaflor, spirotetramat, and chlorpyriphos.

What it means for farmers: We have confirmed that lambda-cyhalothrin resistant aphids in Iowa. We are working to determine if these aphids are also resistant to other insecticides.

Objective 2b. Evaluating potential mechanisms for pyrethroid resistance. We will first focus on metabolism/detoxification of the pyrethroids and secondly address the penetration/uptake of pyrethroids. Aversive behavior or repellency will also be investigated. Objective 3 will include a study of possible gene mutations in the aphid, especially ones that may code for an altered sodium channel, which could be insensitive to the pyrethroids. Metabolism and penetration/uptake studies will employ topical applications of a pyrethroid, followed by a time-course for the experiment, with rinsing, grinding, and extracting the aphids, using a non-polar solvent (ethyl acetate). Determination of the percentage of dose that entered the aphid body (after the external rinse) will be accomplished by GC/MS. Analysis of the solvent extract of the treated aphid bodies will also use LC/MS to measure the parent insecticide and scan for any metabolic breakdown products.

UPDATE: The most resistant to lambda-cyhalothrin was used to evaluate for rapid metabolism /detoxication as a mechanism of pyrethroid resistance in soybean aphid using synergistics (Piperonyl butoxide (PBO), S,S,S-Tributyltrithiophosphate (DEF) and triphenyl phosphate (TPP)). The results obtained for the resistant population were compared to those from the lab-susceptible population.

What it means for farmers: There is the potential to manage pyrethroid-resistant soybean aphids with pyrethroids if a synergist is added. Such an addition could allow farmers to prevent outbreaks without having to switch to a different (any potentially more expensive)-active ingredient. Our findings revealed that adding a synergist to a pyrethroid did not improve the efficacy when used against pyrethroid resistant soybean aphids. Because the synergist didn't work, metabolic detoxification is not a part of pyrethroid resistance in the soybean aphid. This suggests that cross-resistance is unlikely to other insecticides with different modes of action. Therefore, based on our data to date, we do not anticipate pyrethroid-resistant soybean aphids to be more resistant to insecticides like chlorpyrifos than susceptible aphids.

<u>OBJECTIVE 3. The genetic basis of pyrethroid resistance and diagnostic molecular assays</u> (Coates)
A 303-million base pair genome was assembled for soybean aphid biotype-4 (Wenger et al. 2017), a project on which Brad Coates (Co-PI) was involved. He also joined a research team that is preparing the biotype-1 genome assembly for publication in collaboration with Rosanna Giordano (University of Illinois). These two genome assemblies will form the basis for methods proposed to rapidly identify genome regions and genes associated with pyrethroid resistance that will be applied for diagnostic purposes in Objective 3b.

Objective 3a. Determine gene mutations. Advances in DNA sequencing technologies now allow researchers to quickly collect unprecedentedly massive amounts of nucleotide data at very low costs and short time frames, and arguably have the potential to transform agricultural sciences. Using genome re-sequencing methods adapted from Coates and Siegfried (2015) and Steele et al. (2015), we will collect and analyze genomic data from three pools of aphids (pyrethroid susceptible, pyrethroid resistant from the lab colony (Objective 1b), and pyrethroid resistant collected from the field). In brief, during Year 1 Illumina HiSeq3000 reads will be collected and mapped to the soybean aphid genome (Wenger et al. 2017), from computational predictions, mutations will be identified that are different between resistant compared to susceptible pools of aphids. This will allow the identification of fixed gene mutations within resistant aphids. Similar research has been completed by co-PI Coates (Coates et al. 2013, 2016; Steele et al. 2015), where genes and genome regions were linked to insecticide resistance in crop pest insects.

UPDATE: Given the resistance to lambda-cyhalothrin identified in Objective 2, we sought to determine any genetic differences among these populations. We used a candidate gene approach to determine if previously described mutations in the voltage-gated sodium channel (VGSC), specifically, if knockdown (*kdr*) mutations were present in individual soybean aphids from each subpopulation. These kdr mutations change the amino acid sequence and are highly correlated with pyrethroid resistance in other insect species.

We designed primers and sequenced part of segment VI of domain II of the soybean aphid VGSC gene, successfully amplified, and gene fragments DNA sequenced. Comparison of VGSC DNA sequences from susceptible and resistant aphids showed the presence of a *kdr* mutation only in the Sutherland (Iowa), MN1, and MN2 resistant (RES) populations (Fig. 1). In the case of soybean aphids, all pyrethroid resistant individuals were heterozygous for cytosine (C) and tyrosine (T) nucleotides which is predicted to cause a change to the Phenylalanine (F) amino acid in the T allele. These procedures successfully identified a mutation associated with pyrethroid resistance in soybean aphid, but due to differences in resistance ratios in the MN1 and MN2 colonies compared to Sutherland, IA, there likely may be other mutations that contribute to observed resistance phenotypes.

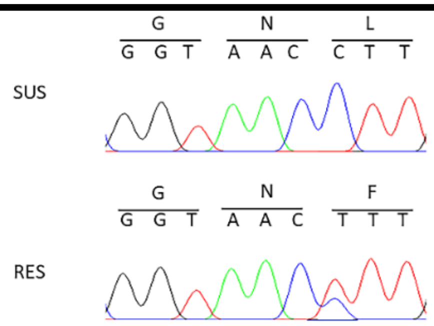

Figure 1. Sequence chromatogram for individual soybean aphids from a susceptible population (SUS) and a resistant population (RES) with the L1024F mutation. Two peaks (representing both C and T) overlap in the RES population, revealing a heterozygous mutation consistent with the *kdr*-mutation.

What it means for farmers: We have found evidence in the genome of the soybean aphid that a mutation is responsible for pyrethroid resistance. Such a mutation can help explain how aphids are resistant and be used as a marker to identify insecticide-resistant aphids in the field.

3b. Develop a diagnostic tool that identifies pyrethroid resistant aphids. Since pyrethroid-resistant and susceptible aphids cannot be visually differentiated, the ability of farmers to make informed decisions regarding which products to apply remains a challenge. Knowledge of the geographic area affected by pyrethroid resistance will greatly assist in these management decisions. Although resistance monitoring can be accomplished using laboratory bioassays (Objective 1a), such methods are expensive in terms of time, labor, and overall costs – in addition to posing health risks to lab personnel. Molecular genetic assays allow researchers to quickly and cost-effectively determine resistance status of insects when causal nucleotide mutations are known. For example, Co-PI Coates developed a DNA-based laboratory assay that successfully determined insecticide resistance status of western corn rootworm and was applied to estimate frequencies in the field (Wang et al. 2013). Although many unknowns exist, application of analogous methods are proposed in this project, where we aim to correlate specific mutations identified in Objective 3a with pyrethroid resistance in the aphid colonies (Objective 1b) and develop diagnostic tools for screening field-collected aphids from Objective 1a.

UPDATE: We furthermore predicted that the C to T mutation between putative resistant and susceptible alleles could be differentiated by digestion of a PCR product with the restriction enzyme *Bst*EII (recognition sequence 5'-GGTNACC-3'; i.e. susceptible gDNA digested but resistant gDNA not digested). To test this, the PCR product from each individual was digested with *Bst*EII overnight and run on a 2-3.5% agarose gel to detect differences between lab susceptible (SUS) and resistant field-collected (RES) populations. Digestion of the PCR product with *Bst*EII yielded fragments of size 154bp and 285bp in the lab (SUS) populations. In contrast the RES populations were heterozygous and produced bands of size 154bp, 285bp (susceptible allele) and 439bp (resistant allele). These results confirmed success of the PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) marker. Due to presumed dominance of the 1024F encoding resistant allele, clonal heterozygotes from the RES population would be resistant.

What it means for farmers: Preliminary evidence suggests that the kdr gene can be used a marker to more quickly identifying insecticide-resistant soybean aphids. This will speed id-work from days to hours.

OBJECTIVE 4. Extension (Hodgson)
In an effort to disseminate the research outputs from Objectives 1-3, a dedicated extension program will be an important component of this project. Erin Hodgson and an extension program specialist will help translate the findings, including creating publications and presentations summarizing resistant issues and promoting management recommendations. Encouraging scouting, use of the economic threshold (Ragsdale et al. 2007), and integrating other IPM tactics will be the focus.

Expected Outputs:

- Objective 1: produce a map with confirmed pyrethroid resistance in Iowa.
- Objective 2: understand the mechanism of resistance for soybean aphid.
- Objective 3: develop a diagnostic tool for timely treatment decisions of resistant aphids.
- Objective 4: provide farmers with recommendations to protect yield and minimize input costs.

Realized Outputs:
1. Objective 1: There were no reported performance issues with any insecticides and soybean aphid during the 2017 summer. However, we collected soybean aphids from a few commercial fields and research plots from northern Iowa in 2017. Pyrethroid resistance was confirmed in O'Brien County again in 2017, in addition to counties within Minnesota, North Dakota, South Dakota and Manitoba.

2. Objectives 2 and 3. A new Ph.D. student, Ivair Valmorbida, started in the Entomology Department in January 12018. He is co-advised by Matt O'Neal and Erin Hodgson. Ivair has already made great strides to develop a literature review, and establish lab and field protocols to complete these objectives. In addition, we have maintained a few soybean aphid populations to begin refining research protocols. The biotype 1 genome assembly was improved using BioNano optical mapping procedures, which resulted in the nearly 6-fold increase in median genome fragment size; from 0.46 to 5.84 million base pair. Small artifactual nucleotide insertions were corrected within the assembly, which allowed for finalizing of the annotated gene models. A series of manuscripts are in preparation for the journal *Insect Biochemistry and Molecular Biology*, with target submission for June 2018. Preliminary genome resequencing data was collected from putative pyrethroid resistant soybean aphids from sprayed fields near Sutherland, IA, which will be compared to analogous resequencing data from susceptible lab colony individuals.

3. Objective 4. Erin Hodgson and members of her lab were active in creating and disseminating research-based management recommendations for a variety of extension audiences. She also is mentoring a current graduate student, Ashley Dean, to develop extension skills to help meet the goals of this objective.

- Erin and Ashley delivered 24 presentations related that focused on soybean aphid management that reached over 1,650 Iowans.
- Erin co-authored a regional publication providing best management practices for soybean aphid in the upper Midwest.
- Erin wrote two proceedings for ISU Extension events.
- She authored a peer-reviewed extension article that showed significant learning of soybean aphid pyrethroid-resistance using a novel evaluation method.
- In 2017 and 2018, Erin summarized foliar efficacy evaluations for soybean aphid in a departmental publication and peer-reviewed publication.
- Erin was the lead editor for a revision of the NCSRP regional field guide for soybean aphid management with Dr. Robert Koch, University of Minnesota.
- Erin created 1 video that was distributed to the ISU Commercial Applicator Program.

Realized Stakeholder Deliverables and Outcomes:
Matt O'Neal and Erin Hodgson organized a resistance management field day in August 2017 to raise awareness of pyrethroid resistance. Outcomes from the field day:

- Understanding SBA life cycle and biology had a 1,100% percent increase.
- Knowing SBA host plant resistance options had a 900% percent increase.
- Awareness of SBA insecticide resistance had a 700% percent increase.
- Implementing integrated SBA management had a 1,000% percent increase.
- Participants were asked about their perception of value, and 100% said it was a "very valuable" workshop. There were notable behavior changes indicated in the survey summary: 58% said they will consult with ISU regarding future pest management decisions and 44% said they will scout and use economic thresholds for SBA.
- In general, respondents indicated the four speakers contributed to a successful workshop. For example, 93% of people attending the workshop said Erin Hodgson had "excellent" energy, and 100% of people said Erin had "excellent" engagement with the group and were experts in their respective fields.