Soybean entomology in the North Central region:
Management and outreach for new and existing pests

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Project Objectives

I. Extension and Outreach

II. Insect Monitoring and Management
1. Stink bug monitoring and management
2. Pollinator diversity and soybean yield
3. Soybean aphid insecticide resistance
4. Monitoring for aphids, thrips, and soybean vein necrosis
5. Technology development

III. Resistant Varieties and Biotypes
1. Breeding for resistant varieties
2. Aphid virulence genotyping and mapping
3. Aphid virulence management for resistant varieties
4. Economic returns on resistant varieties

IV. Biological Control

Progress Report (Year 3, October 2017 through March 2018)

I. Extension and Outreach

We completed the field guide Stink Bugs of the North Central Region and distributed 6,000 hard copies to the 12 states in the region, with a supply sent to state extension specialists and to each of the state checkoff boards. A pdf has been made available to SRII and other online sites for free electronic distribution.

II. Insect Monitoring and Management

1. Stink bug monitoring and management: The goal is to devise management thresholds for stink bugs that are specific to the North Central Region. Progress during the reporting

period has focused on processing samples, summarizing and analyzing data: From mid-July through late September in 2017, 62 soybean fields from 9 states were sampled weekly using sweep nets. From 381 sets of 25 sweeps, a total of 2,004 individuals of 18 phytophagous stink bug species were collected, the most abundant adults and nymphs were the green stink bug (Chinavia hilaris) and brown stink bugs (Euschistus spp.). The spatial pattern of plant feeding stink bugs was assessed using Taylor's Power Law (TPL) and sampling plans were developed and validated using the Resampling for Validation of Sample Plans (RVSP) software based on Green's method with data from 2016 and 2017. Moreover, preliminary analyses of the influence of landscape factors and within-field factors were performed on data from 2016. For that, we used the CropScape and Cropland data layer on ArcGIS to characterize and quantify habitats surrounding soybean fields. Principal component Analysis (PCA) were performed to reduce the complexity of the landscape data and linear regression models to test the effect of tillage, seed treatment, latitude, longitude and PCA results on stink bug density. Stink bug diversity and abundance results and landscape and within-field factors results were presented at the Regional (2018) and National (2017) Entomological Society of America (ESA) meetings, respectively. Data from the two years have been entered into spreadsheets and are being analyzed. Manuscripts are being drafted on these results.

2. Pollinator diversity and soybean yield: The goal of this study is to document the diversity of pollinators present in soybean fields. Pollinators may enhance soybean yield, and soybean may serve as an important reservoir for pollinator biodiversity. Participants in ND, SD, IA, OH, MN, NE, IN, MO, WI collected pollinators in soybean fields in Year 1 of the project. Identifying all the bee species collected took longer than anticipated but during the reporting period identification was completed. We were positively surprised by the overall bee and syrphid fly species richness and abundance in the surveys.
108 bee species in 27 genera from five families, and 11 syrphid fly species in six genera Syrphid flies and especially bees can be relatively abundant and diverse in soybean fields, a monoculture environment. Our results provide baseline data on the bee and Syrphid fly fauna associate with flowering soybean in Midwest.

   The other component of this objective is to assess the diurnal activity of wild and managed bees in soybeans. Knowing the time of day when pollinators are most active in soybean can help producers make decisions about when to spray. During the reporting period we assessed data from the summer of 2017 and decided to develop a new protocol because the methodology in 2017 was not good enough. We are currently preparing for the 2018 field season with a revised bee collection method.

3. Soybean aphid insecticide resistance: The goals of this objective are to monitor for soybean aphid resistance to the insecticide thiamethoxam in the North Central Region. Results of analysis of several years of data (reported in previous progress reports) were published in the Journal of Economic Entomology. Results were included in presentations at the UNL Crop Production Clinics. Protocols were optimized for the 2018 field season. Supplies were purchased. Post-doc assigned to the project resigned and the

project was assigned to an existing technologist (Terence Spencer) in the University of Nebraska-Lincoln Insect Toxicology Laboratory under the supervision of Dr. Ana Velez.

4. Monitoring for aphids, thrips, and soybean vein necrosis: Soybean vein necrosis virus is transmitted by thrips. We sampled thrips from suction traps in 6 states. We have completed thrips survey for the 2017 growing season, and begun identifying thrips to species from 2016 and 2017 seasons. Thrips population dynamics was very similar in 2016 and 2017. Thrips populations start to increase as early as June which coincides with early vegetative stages of soybean in most Midwest states. Thrips activity peaked in July-August in most states and begins a decline in September. Recently, we began identifying thrips to species, and preliminary results indicate that flower thrips is the most predominant thrips in most locations followed by soybean thrips; tobacco thrips is also present throughout the growing season. The three confirmed thrips vector of SVNV, soybean thrips, flower thrips and tobacco thrips were detected in all locations, which suggest that there is potential for SVNV outbreaks in these regions.

   For the sub-objective of suction trap monitoring for soybean aphid population trends, during the reporting period, most of the suction trap supplies have been ordered. Packaging the supplies to 29 locations is in progress (Champaign, and Ames, require supplies to be mailed). We expect to ship all the supply packages to our collaborators by the first week of May. The plan for this coming season is to operate the suction traps from May 18 through October 19, 2018 (23 weeks). The trap located in Orr, IL will not operate due to the lack of collaborator, and the research station is closed. A trap will be relocated to Freeport, IL. The traps located in Bean and Beet, MI and Eau Claire will resume operation this year due to a request from extension personnel in those locations. Suction trap data were shared with Joseph LaForest from University of Georgia, who is part of the team of the “Center for Invasive Species and Ecosystem Health” (commonly known as the “bugwood network”). The data have been entered and cleaned up (mainly misspelling of scientific names). The future data collected from the Midwest Suction Trap Network will be entered straight to “bugwood” and this information will be available online to the suction trap collaborators, farmers and extension personnel. The website has been named as the “Suction Trap Network” Also, suction trap data was shared with Nick Seiter from University of Illinois, who will use past and future data for outreach purposes.

5. Technology development: The goal is to develop an aphid-counting app. Performance indicators for smartphone-based estimation algorithm included a refinement of the original sampling algorithm, collection of additional images (approx. 6400) from different device types (3 total). All aphid pictures captured during this project were manually counted, processed and statistically analyzed. The following factors were compared within and across four fields in two states (Iowa and Minnesota): leaflet position (newly expanded node, and mid- and low-canopy leaflets), sensor type, sensor distance from leaflet (8 to 12 cm), and background color (white, black, and green). Both sensor type and sensor distance varied slightly within fields, but responses were inconsistent between states; aphid density was low in Iowa and higher in Minnesota. In general, our algorithm grossly underestimates aphid counts when under high densities

(>50 per leaflet) but overestimates when aphid density is low (<10 aphids per leaflet). Overall, background color and distance of the sensor from the leaflet did not influence accuracy of estimations. Next steps include testing the affect of aphid density on accuracy and revising the algorithm to adjust counts based on low or high aphid estimates using unsupervised machine learning techniques and other open-source software used for quantitative analysis of biological images.

III. Resistant Varieties and Biotypes

1. Breeding for resistant varieties: The Diers program is developing and releasing soybean varieties with aphid resistance. During the past six months, research has been conducted to backcross Rag4 and Rag6 into the same MG I and MG II backgrounds that Rag1-3 were previously backcrossed. We now completed the backcrossing of both Rag4 and Rag6 into the two backgrounds, and combined Rag4 with Rag1-3. Plants that have Rag1-4 were selected in the two backgrounds in the greenhouse this spring. Crosses are now being made between these selected plants and plants heterozygous for Rag6. If this crossing is successful, we will have in the field this summer plants that are heterozygous for Rag1-4 and Rag6. Lines with all combinations of these genes will then be selected for use in future research. In related work in resistance screening in the Hesler lab, two papers were submitted for peer-review journal publications. The papers summarized results of two studies to evaluate wild soybean lines for resistance to an avirulent soybean aphid biotype. As a follow-up, further experiments evaluated resistant lines from those two studies and resistant soybean lines against soybean aphid biotype 4, which is virulent to both Rag1 and Rag2 resistance genes. The additional experiments were carried out using three iso-female colonies of soybean aphid biotype 4. Two wild soybean lines and one cultivated soybean line were found resistant to biotype 4, and these three lines should be explored further as potentially valuable sources of soybean aphid resistance.

2. Aphid virulence genotyping and mapping: Our goal is to map aphid virulence. We have explored hypotheses to explain the segregation distortion seen in crossing soybean aphid biotypes. We have found evidence of hybridization among the soybean aphid and Aphis nasturtii, a related aphid that shares the mating and overwintering host (buckthorn). We have compared the genetics of Wolbachia, a bacteria present in some insects, including the soybean aphid, that sometimes causes sterility. Based on our initial analyses, the Wolbachia species found among soybean aphid biotypes are similar. Another possibility for the segregation distortion could be hybridization with Aphis nasturtii, a related aphid that shares the same overwintering host. We compared genetics of these two species and did find similarities that might result from hybridization. We will generate additional data to test this hypothesis from buckthorn collections in the spring of 2018.

3. Aphid virulence management for resistant varieties: We have determined that adding parasitoids to microcosms with resistant plants only may negatively impact survival of biotype 1. There did not seem to be an impact of parasitoids on susceptible plants, suggesting that adding a refuge to HPR may positively benefit insect resistance management. We conducted a field experiment to test whether a refuge-in-a-bag approach can protect yield while maintaining a population of soybean aphids. In a field

experiment conducted at three states (Iowa, South Dakota and Ohio), we evaluated the effects of (a) host plant resistance (Rag), (b) inclusion of a susceptible refuge blended with a resistant variety, and (c) foliar insecticide treatment on aphid population and yield. Insecticide treatment was included to estimate yield loss from aphids and genetic differences between resistant and susceptible plants. Results: Aphids colonized plants from late July through September. All treatments that included aphid-resistant seeds were not significantly different from each other. These results indicate that inclusion of a susceptible refuge did not affect the capacity of Rag genes to limit aphid outbreaks, but did maintain an aphid population consistent with refuge requirements. When averaged across all refuge treatments, insecticide treatment also had an overall significant impact on soybean yield and provided a nearly 2 bushels/acre yield boost. However, because of the low aphid pressure experienced in 2017, we suspect the yield loss was not caused by soybean aphids but rather by some other insect(s) controlled by the insecticides. While insecticides had an overall effect on yield, our aphid-free environment results provide evidence that aphid resistance genes Rag1 and Rag2 genes do not cause yield drag. What does it mean for farmers: Overall, our data suggests that aphid-resistant soybeans blended with aphid-susceptible plants could serve as a refuge while still being effective for suppressing aphids in the field. Thus, refuge-in-a-bag could be a viable resistance management strategy for the sustainable use of soybean aphid resistant soybeans.

4. Economic returns on resistant varieties: This is a three-summer study designed to assess the economic returns on herbicide tolerant and aphid resistant traits. Although yield is still slightly inconsistent among different environments, aphid-resistant plants showed consistent protections from aphids and remain a valuable, cost-effective tool for aphid management. We tested these three hypotheses: 1) aphid-resistant varieties provide protection from soybean aphids; 2) aphid-resistant varieties are more valuable when many aphids are present; and 3) host plant resistance is more valuable than insecticides. We used two aphid environments, two yield environments and four soybean varieties. Varieties with host plant resistance had fewer cumulative aphid days. Host plant resistant varieties offer consistent protection from aphid exposure. Soybean yield is equivalent to an insecticide application where aphids are abundant . Our study shows that there may be some room for improvement in these particular varieties to provide consistent yields across the four varieties in all yield environments.

IV. Biological Control

We continued studies on overwintering biology of Aphelinus certus and A glycinis. Diapausing mummies of both species were placed into various field settings: open soybean field, soybean field with leaf litter, leaf litter in a wood lot and suspended on buckthorn in a wood lot. Each of these treatments was replicated four times with 300 mummies per replicate. These mummies were brought into the lab for emergence in mid-April and emergence is currently being monitored. In a separate lab study we determined the effect of host density on the longevity and lifetime reproductive success of Aphelinus certus. This study showed that the parasitoids die in a few days if given only one aphid per day but that they can live for about 3 weeks if given 10 or

50 aphids per day. This increase is presumably due increased opportunities for host-feeding and/or honeydew feeding. The average number of aphids parsitized in the 50-aphid/day treatment was 340.