08-2019, Using weeds as a resource to develop herbivore-resistant soybean
Final Report

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RATIONALE/JUSTIFICATION FOR RESEARCH:

White-tailed deer are responsible for 70% of the wildlife caused crop losses totaling $4.5 billion in crop losses each year (Miller et al. 2015). Soybean plants protected from white-tailed deer browsing were 25% taller, 87% less damaged, yielded 74% more seed, and had 47% more above-ground biomass than unprotected plants in year 1 and 2 of this project. A loss of $68/ha or a 43% financial loss over one growing season to deer browsing is estimated (Conover 2002). Unfortunately, the only effective and widely used technique to control deer in soybean currently is establishment of fences or application of repellents (Cauteren et al. 2006). Fencing is expensive and labor intensive to install, and require weekly inspection and maintenance throughout the growing season to ensure effective operation and longevity (Ward et al. 2010). Effectiveness of repellents is also reduced by rainfall that may dissolve repellents requiring reapplication and are not affective against deer. Moreover, soybean insect pests, especially soybean aphids, have the potential to reduce soybean yield by up to 41% and the number of pods per plant by up to 40%, when present at the R2 stage at a density of 100 aphids/plant. There have been few cultivars released with soybean insect resistance, however, none of them are widely accepted by the growers because of its inadequate resistance levels, poor agronomic characteristics, and inferior seed yield.

Plants possess varying levels of herbivore defense mechanisms, and weeds, because of their vast genetic and phenotypic diversity, are a good resource for anti-herbivore traits (especially against deer, and insect pests). No one has tried to test the activity and effectiveness of these anti-herbivore compounds or plant extracts on crop protection. In Year 1 & 2, we conducted tests at the Mississippi State University (MSU) Captive Deer Facility and at The R. R. Foil Plant Science Research at MSU to confirm the anti-herbivore property of sicklepod weed extracts. Sicklepod extracts were tested by application on soybean plants in a diet selection trial on captive deer and insects. In our trials, soybean plants without sicklepod extract were consumed completely, while plants with sicklepod extract were entirely avoided. In field trials excluding all herbivores, application of sicklepod extracts did not cause any soybean yield reduction compared to untreated plants thus indicating the sicklepod extract has no adverse effect on overall soybean yield. Insect damage was apparent in our treatment area and our data suggests that soybean plants treated with sicklepod extract had lesser insect damage than two other insect repellants available in the market. In Year 1, we also quantified the amount of three anthraquinone derivatives in sicklepod and soybean, using high performance liquid chromatography (HPLC), and found the levels of these anthraquinone derivatives (chrysophanol, emodin, physcion) to be up to 11 times higher in sicklepod compared to soybean. In Year 2, we screened 50 core accessions of wild soybean (USDA Soybean Germplasm Collection) and identified three accessions tolerant to deer browsing, with WS22 being the most tolerant among the three. Extracts of these three wild soybean accessions, together with sicklepod and soybean, were analyzed for anthraquinones (deer repellant compounds identified in Year 1 & 2 of this project) in leaf tissues, and wild soybean accessions showed similar or higher anthraquinone content (emodin, chrysophanol, and physcion) as sicklepod; thus suggesting wild soybean accessions to be as effective as sicklepod in repelling deer. Additionally, HPLC and TLC results indicate the presence of other unknown compounds (apart from anthraquinones) that are present in higher amounts in wild soybean than soybean and sicklepod, thus suggesting that there may be additional compounds associated with deer and/or insect repelling property in wild soybean. Thus, in Year 3, we identify additional unknown compounds other than anthraquinone that may be significantly associated with deer and/or insect repelling property, and a combination of column chromatography and NMR spectroscopy will help achieve this.

Column Chromatography (CC) has the capability to handle large mass/volume processing and to divide extract into distinct fractions. Large-scale chromatography columns are readily designed to meet a wide variety of compound separation by using appropriate materials for construction. CC may thus offer an alternative to HPLC for the precise separation and purification of additional anti-herbivore compounds in wild soybean and sicklepod. Nuclear magnetic resonance (NMR) spectroscopy is another powerful technique for identifying and obtaining detailed structural information about organic compounds in plant extracts. Based on the molecular mass, NMR spectroscopy has the ability to provide additional information to precisely identify a completely unknown compound (Albert 1995). Understanding the biochemical pathway or genes associated with the production of these anti-herbivore compound(s) in wild soybean will enable us to select this trait in soybean. Molecular markers can be developed associated with these pathway/genes, and used in screening soybean germplasm for the anti-herbivore traits, or use it in molecular breeding to breed the anti-herbivore trait from wild soybean into soybean cultivars. Soybean with significant anti-herbivore property in leaves will prevent yield losses incurred due to yield-reducing insect pests, and herbivores such as deer. Moreover, anti-herbivore properties can be extracted from sicklepod and applied on soybean to protect the crop from deer and yield-reducing soybean insect pests such as soybean aphids, bean leaf beetles, threecornered alfalfa hopper, green cloverworm, soybean looper, and stink bugs.

OBJECTIVES (Year 1, 2, and 3):

(1) Prepare plant extracts of potential weed species and develop a liquid formulation for application on soybean crop;
(2) Conduct field trials using unmanned aerial vehicles (UAVs), plant surveys, and trail cameras to quantify herbivore use and damage to soybeans in treatment and control plantings;
(3) Conduct chromatography and mass spectrometry analysis to identify target anti-herbivore compounds in weeds; and,
(4) Conduct quantitative trait loci analysis to identify molecular markers associated with anti-herbivory compounds in weeds.
(5) Screen diverse germplasm of soybean primarily consisting of wild accessions and some commonly grown cultivars in the southern US, for high anthraquinone production;
(6) Select high anthraquinone soybean accessions (from objective #1) for trials in the captive deer facility and research fields to quantify deer and insect use and damage to soybeans in treatment and control plantings; and
(7) Conduct quantitative trait loci (QTL) analysis to identify molecular markers associated with anthraquinone related genes in soybean.
(8) conduct C4 column chromatography to separate sicklepod and selected wild soybean extracts into four fractions;
(9) use the four fractions (from objective #1) for trials in the MSU captive deer facility and research fields to determine which fraction(s) is most effective in repelling deer and insect; and
(10) identify specific compounds in fraction(s) (from objective #2) putatively associated with deer repelling property, using NMR spectroscopy and Gas Chromatography/Mass Spectrophotometry (GC/MS) analyses.

REPORT OF PROGRESS/ACTIVITY:

Objective 1: As chrysophanol, emodin and physcion were listed as main anthraquinone components in sicklepod seeds and roots, they were selected as standards to estimate anthraquinone derivative concentrations. Two extractions were adopted: *Extraction 1* was used for qualitative analysis; and, *Extraction 2* was used for quantitative analysis; all quantitative analyses were conducted in three replicates.

Extraction 1: Plant part powder of 0.10 g was weighted into a 1.5 mL tube, followed by the addition of 1 mL methanol and 200 μL chloroform. The tube was mixed for 20 min and centrifuged at 13,200 rpm for

10 min. The resulting supernatant was filtered (0.2 μm pore size) and air evaporated overnight for HPLC and TLC analyses.

Extraction 2: The sicklepod leaf powder of 1.0 g was weighted into a 50 mL tube, followed by the addition of 20 mL methanol, 5 mL double deionized water, and 5 mL chloroform. The tube was mixed for 4 hours and vacuum filtered. Ten ml of methanol was used to rinse the plant powder in the Buchner Funnel. The combined filtrate was evaporated to dry in water bath at 60 °C, and then 3 mL methanol was added to dissolve for HPLC and TLC analyses.

Objective 2: Field plots were established in RR Foil Plant Science Research Center (North Farm) on June 2017 to determine the efficacy of different deer repellent treatments on deer. The various deer repellent treatments were, sicklepod root extract, three commercially available deer repellents, and water to serve as control. Aerial vehicles (UAVs) and plant responses such as % injury, % stunting, chlorophyll content, and % browsing are being evaluated/recorded on weekly basis. In our Captive Deer Facility trials, soybean plants without sicklepod extract were consumed completely, while plants with sicklepod extract were entirely avoided (Figure 1, Figure 2). In field trials excluding all herbivores, application of sicklepod extracts did not cause any soybean yield reduction compared to untreated plants thus indicating the sicklepod extract has no adverse effect on overall soybean yield (Figure 3). We used imagery from 2016 to determine where to establish our field trials by targeting high-herbivory areas, but our treatments resulted in a shift in deer herbivory to another part of the field (Figure 4). Thus, we did not observe a difference between controls and treatments because deer avoided the entire area. The field experiment is currently being repeated at Andrew's Forestry and Wildlife Experiment station which has high deer density. We will also repeat the field experiment next year, at no cost, by conducting whole field treatments to quantify the change in deer herbivory on entire field production. Insect damage was apparent in our treatment area and we data suggests that soybean plants treated with sicklepod extract had less insect damage than our control or two other formulations on the market (Figure 5). Results from the captive deer experiment demonstrated that anthraquinone extracts from sicklepod applied on soybean is able to repel deer and prevent insect feeding. Furthermore, in the field experiment, when all herbivores were excluded we demonstrated that sicklepod extract application did not affect soybean yield.

Figure 1. Comparison of deer browsing of soybean plants applied with sicklepod extract (A), and applied with water as control (B). About 95% of the vegetation of soybean plants applied with water was browsed by deer, while none of the vegetation of soybean plants applied with sicklepod extract was browsed.

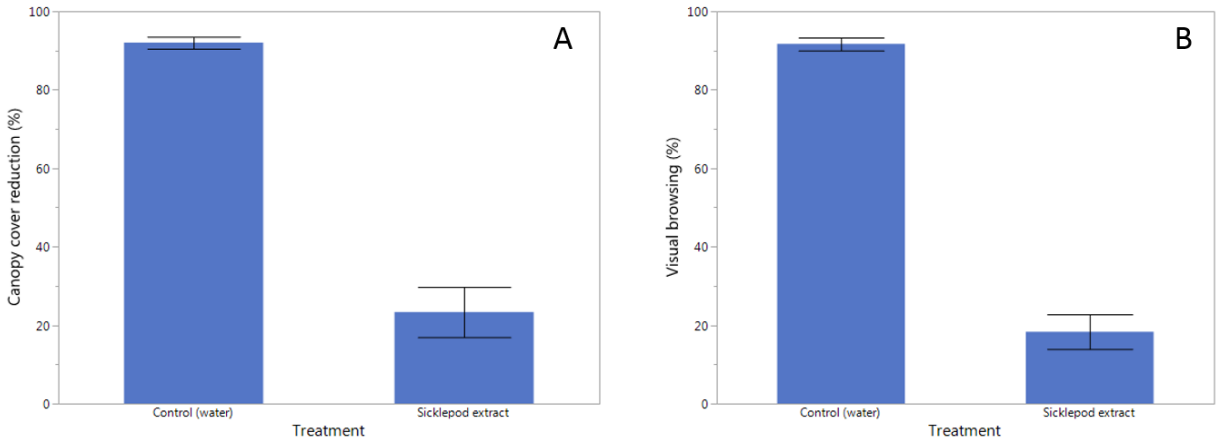


Figure 2. Canopy cover reduction (%) (a), and visual browsing (%) (b) of soybean seedlings treated with water (control), and sicklepod seed extract, after 4 hr of exposure to captive deer at the Captive Deer Facility at Mississippi State University. Images of soybean canopy were captured before and after deer exposure and analyzed using image analysis software Image J to determine percent canopy cover reduction caused by deer browsing. Visual browsing was recorded on a scale of 0 to 100% where 0 = no browsing, and 100 = completely browsed.

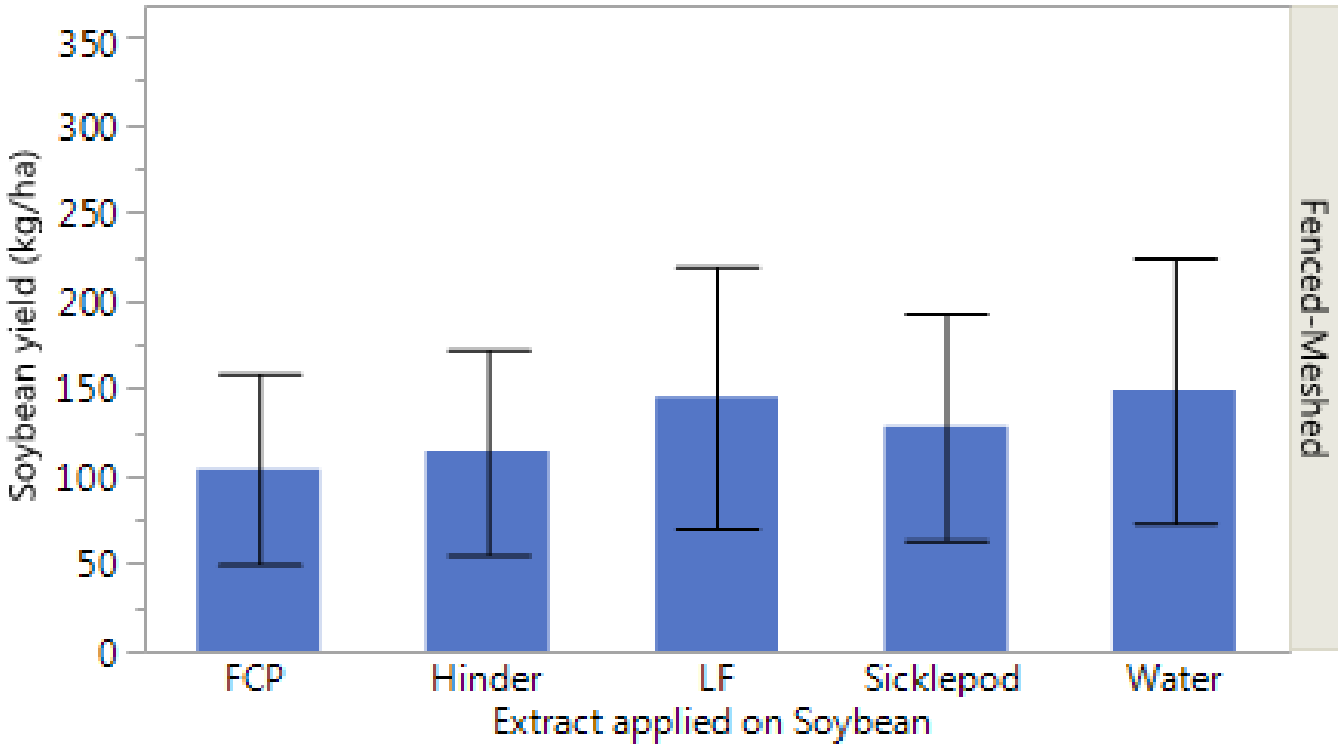


Figure 3. Effect of application of deer-repelling extracts on soybean yield. All treatments were under electric fencing and covered using nylon net (10 mesh/in) to protect plants from deer and insect damage, respectively. FCP (Flight Control Plus), Hinder, and LF (Liquid Fence) are commercially available herbivore-repellents (primarily deer), sicklepod is the extract of sicklepod plants, and water serves as an untreated control.

Figure 4. Aerial photograph of white-tailed deer herbivory damage from UAV flights. We targeted our treatment area (black solid line) in the high deer herbivory area from 2016 (red dotted line) and deer herbivory shifted to avoid our treatments entirely in 2017 (red solid line).

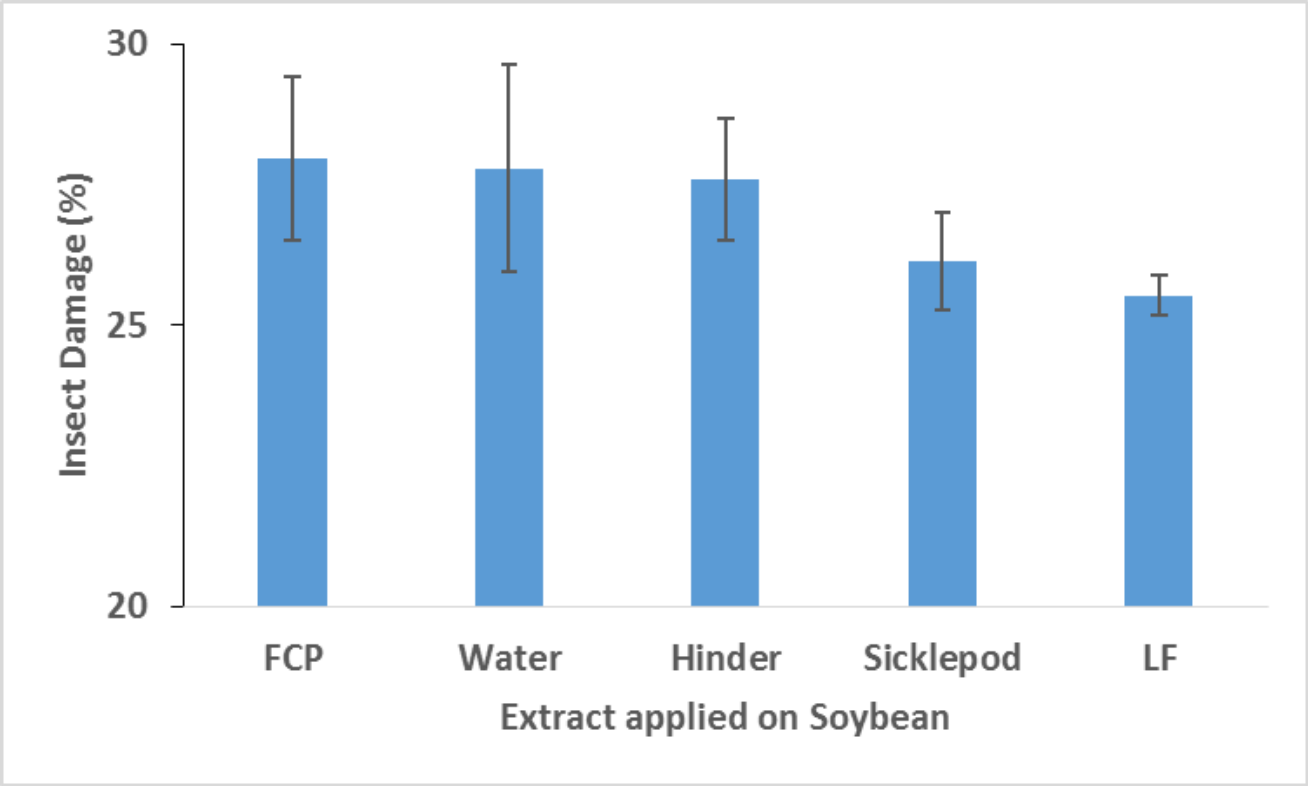


Figure 5. Effect of application of deer-repelling extracts on soybean insect damage. FCP (Flight Control Plus), Hinder, and LF (Liquid Fence) are commercially available herbivore-repellents (primarily deer), sicklepod is the extract of sicklepod plants, and water serves as an untreated control.

Objective 3:
High pressure liquid chromatography (HPLC) analysis: An Agilent 1100 series HPLC (Agilent, Santa Clara, CA) was used to analyze anthraquinone derivatives in the extracts. The HPLC consisted of tandem diode array (DAD) and fluorescence (FLD) detectors, an online vacuum degasser, a quaternary pump, an autosampler and a thermostatted column compartment. The Agilent Chemstation A.10.02 software with a spectral module (Agilent Technologies Inc., Wilmington, DE, USA) was used to process the data. Separation was achieved on an Alltech Adsorbsphere reverse phase C18 column (150 mm x 4.6 mm, Dr. A. Maisch High Performance LC GmbH, Germany) with particle size 3 µm. Anthraquinone derivatives were detected at 254 nm (Dionex, 2009), with a flow rate of 1 mL/min and column temperature of 30ºC. Peaks were identified using standard compounds (chrysophanol and emodin). The injection volume of 5 µL was used. Gradient elution program was developed as follows: Eluent was mixed by acetonitrile with water. Acetonitrile keeps 40% for the first 8 minutes, followed by increasing to 70% from 8 minutes to 21 minutes, then keeps at 70% to 25 minutes. Sicklepod plant part extracts (stem, root, fruit and leaf) from *Extraction 2* were analyzed for chrysophanol and emodin.
UV Fluorescence of Anthraquinone derivatives: A fluorescence microscope (Olympus BX51) equipped with a cube consisting of filters D360/40x, 400dclp, and ET560lp, was used to observe the fluorescence of the anthraquinone derivatives. The latter filter completely blocks the UV excitation (350 nm) and allows only signals from approximately 560 nm and longer to be passed. The UV excitation of the microscope was 350 nm. Such setting allowed the microscope to observe fluorescence of anthraquinone

derivatives around 580-600 nm (yellow). Fresh plant tissues (sicklepod root, stem and soybean stem) were cut free-hand using a razor blade into think sections and immediately examined under the microscope. All observations were in three replicates.

Conclusion: The UV fluorescence of anthraquinone derivatives can be used to observe anthraquinone distribution in sicklepod plant using fluorescence microscope. Cell types with anthraquinone levels listed in their descending order: endosperm cell > podshell cell > root cell > stem pith cell (Figure 6). They all have high anthraquinone contents, but endosperm has less cellulose and high galactomannans while other cells are rich in cellulose. Cell cultures of endosperm or podshell (J) may be an alternate method to prepare sicklepod extracts rich in anthraquinone derivatives (Figure 7). Sicklepod fruit contains high anthraquinone concentration with anthraquinone derivatives primarily concentrated in endosperm. The arrangement of the membrane structures rich in anthraquinone derivatives, open structures, and irregular shape, suggest they may not be cell membrane but instead organelle; although, they are similar to cell size. The size and shape of the hollow membrane structures in endosperm (K, L & M) are similar to those of the bright yellow "cells" in podshell (J), thus indicating they may be the same structures (Figure 8).

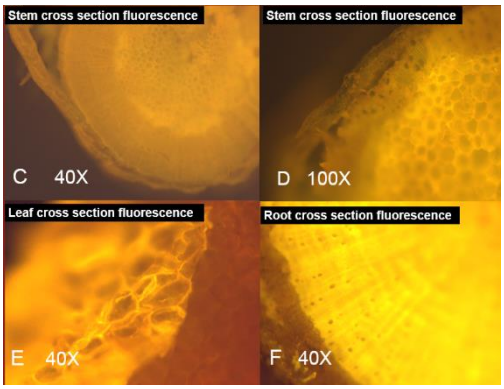


Figure 6. Stem section fluorescence shows three different anthraquinone concentrations: pith > woody stem > stem bark; (D) shows lowest anthraquinone concentration in stem surface; (E) leaf surface is dark while inside is bright yellow (anthraquinone derivatives); the leaf cell on leaf surface is estimated around 40 µm; and, (F) root section with high anthraquinone derivatives (bright yellow); surface cells show lower anthraquinone concentrations.

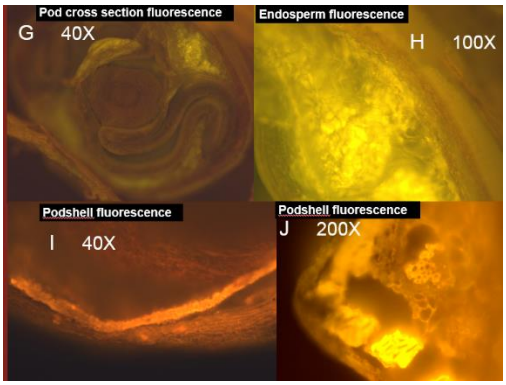


Figure 7. Pod cross-section: the radicle and plumule are relatively dark while the endosperm is bright yellow; (H) shows high anthraquinone concentration in endosperm; (I) shows podshell containing tissues with high anthraquinone derivative concentration (orange); and, (J) also shows some pod shell tissues contains high anthraquinone concentrations (bright yellow). The bright yellow cell section is around 28 µm.

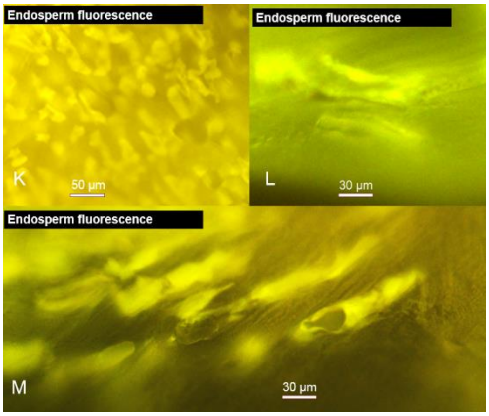


Figure 8. Pod cross-section: (K), (L), and (M) shows hollow membrane structures rich in anthraquinone derivatives in endosperm. They are irregularly shaped (K), and are isolated from each other or floating in the endosperm; 75% of which is galactomannans (Shang et al. 2012); they are not necessarily closed structure (L and M). Their section size is around 28 µm and length is around 55 µm.

Objective 4: Conduct quantitative trait loci analysis to identify molecular markers associated with anti-herbivory compounds in weeds. This objective was modified to more precisely target the genes related to anti-herbivory. Instead of screening the weed (sicklepod) for markers related to anti-herbivory, we plan to screening a large germplasm of wild soybean varieties for high anthraquinone production using HPLC analysis. Soybean varieties having high anthraquinone production will then be used in quantitative trait loci analysis to associate molecular markers to anthraquinone production. This objective will be continued throughout the second year of funding as no additional cost to MSPB.

Objective 5: Screen diverse germplasm of soybean primarily consisting of wild accessions and some commonly grown cultivars in the southern US, for high anthraquinone production. One-hundred soybean accessions representing landraces, wild, and cultivated varieties were selected and obtained from Esther K Peregrine at the USDA Soybean Germplasm, Pathology, and Genetics Research Lab in University of Illinois, Urbana, IL. Only accessions with southern maturity were selected and thus more adapted to the growing conditions in Mississippi. Four plants from each accession were propagated in greenhouse until maturity, after which, plant extracts of each soybean accession were prepared using two different extraction methods. *Extraction 1* was used for qualitative analysis; and, *Extraction 2* was used for quantitative analysis; all quantitative analyses were conducted in three replicates.

Extraction 1: Plant part powder of 0.10 g was weighted into a 1.5 mL tube, followed by the addition of 1 mL methanol and 200 µL chloroform. The tube was mixed for 20 min and centrifuged at 13,200 rpm for 10 min. The resulting supernatant was filtered (0.2 µm pore size) and air evaporated overnight for HPLC and TLC analyses.

Extraction 2: The sicklepod leaf powder of 1.0 g was weighted into a 50 mL tube, followed by the addition of 20 mL methanol, 5 mL double deionized water, and 5 mL chloroform. The tube was mixed for 4 hours and vacuum filtered. Ten ml of methanol was used to rinse the plant powder in the Buchner Funnel. The combined filtrate was evaporated to dry in water bath at 60 °C, and then 3 mL methanol was added to dissolve for HPLC and TLC analyses.

HPLC and TLC results indicate the presence of other unknown compounds (apart from anthraquinones) that are present in higher amounts in wild soybean than soybean and sicklepod (Figure 9), thus suggesting that there may be additional compounds associated with deer and/or insect repelling property in wild soybean. There is thus a need to identify additional unknown compounds other than anthraquinone that may be significantly associated with deer and/or insect repelling property, and a combination of column chromatography and NMR spectroscopy will help achieve this.

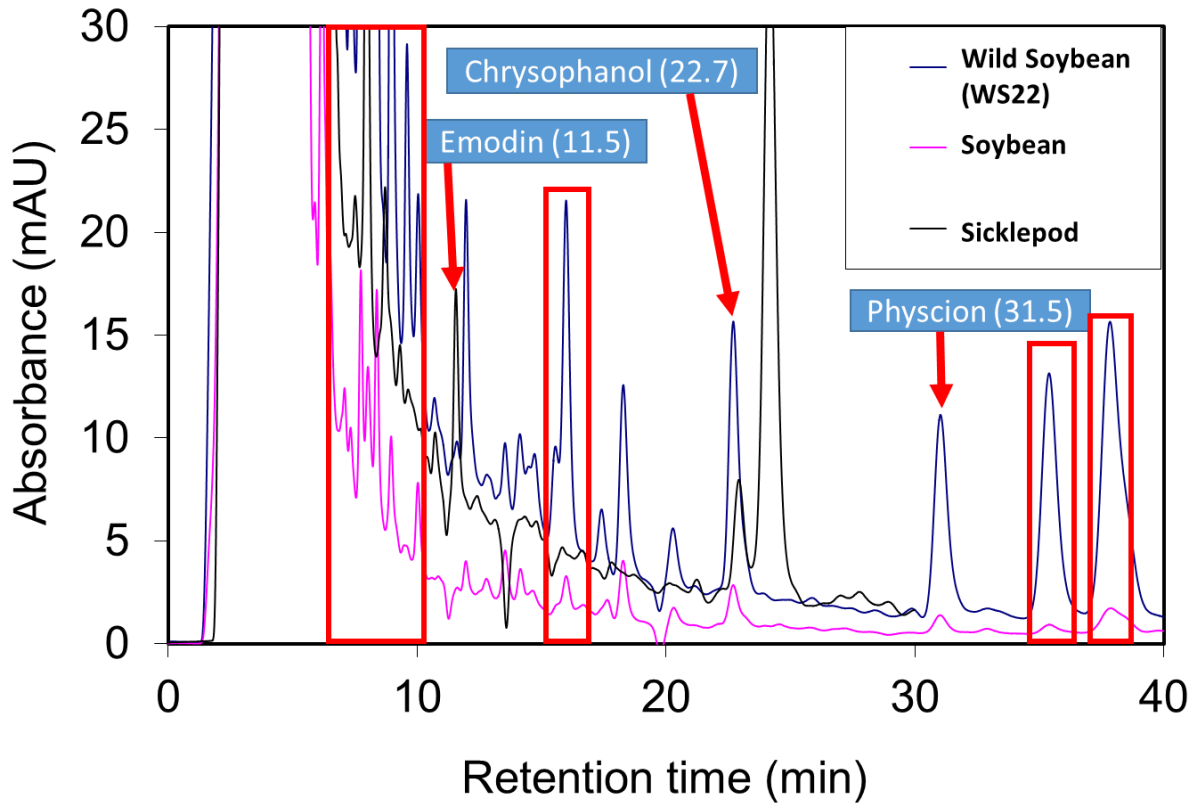


Figure 9: Stacked chromatogram of wild soybean (WS22), sicklepod, and soybean leaf extract showing anthraquinone contents (emodin, chrysaphanol, and physcion). Soybean showest the lowest anthraquinone content, while wild soybean contain equal or higher anthraquinone content than sicklepod. The chromatogram also shows numerous peaks (red box) in wild soybean (WS22) that are higher than soybean, and corresponds to potential deer and/or insect repelling compounds.

Objective 6: Select high anthraquinone soybean accessions (from objective #1) for trials in the captive deer facility and research fields to quantify deer and insect use and damage to soybeans in treatment and control plantings. We screened 50 core accessions of wild soybean (USDA Soybean Germplasm Collection) and identified three accessions tolerant to deer browsing, with WS22 being the most tolerant among the three (Figure 9). Extracts of the three wild soybean accessions, together with sicklepod and soybean, were analyzed for anthraquinones (deer repellant compounds identified in Year 1 & 2 of this project) in leaf tissues, and wild soybean accessions showed similar or higher anthraquinone content (emodin, chrysophanol, and physcion) as sicklepod (Figure 10); thus suggesting wild soybean accessions to be as effective as sicklepod in repelling deer.

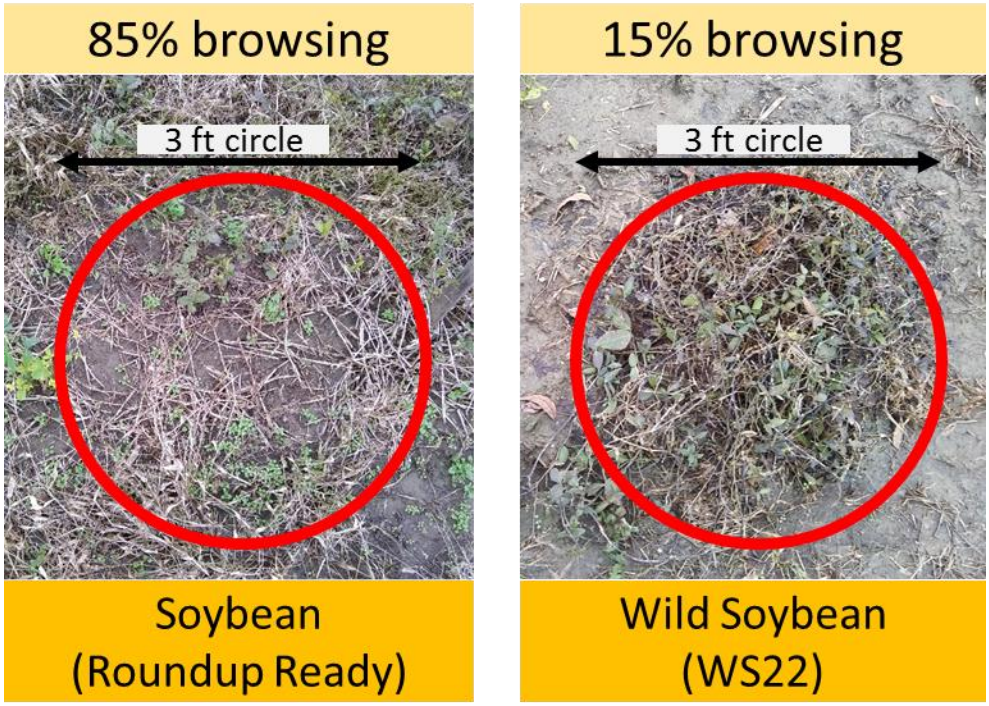


Figure 10: Wild soybean (WS22) with 10% browsing, compared to soybean (Roundup Ready) with 95% browsing, thus suggesting wild soybean (WS22) be effective in repelling deer.

Objective 7: Conduct quantitative trait loci (QTL) analysis to identify molecular markers associated with anthraquinone related genes in soybean. Leaf tissues from each soybean accessions were collected and stored in -80C until used in DNA extraction. Fifteen SSR molecular markers were selected and ordered from IDT DNA. Polymerase chain reaction with these primers are still being conducted to standardize the temperature cycles, and DNA has been extracted from 35 wild soybean accessions.

Objective 8: Conduct C4 column chromatography to separate sicklepod and selected wild soybean extracts into four fractions. ***Crude Extract Preparation****:* Ground plant material was extracted at room temperature for 24 hours using 250 mL of hexane, providing 490 mg of extractables after evaporation of the solvent. Dried sample was subsequently extracted using 250 mL of methylene chloride (DCM), providing approx. 300 mg of extractables following evaporation of the solvents. This process was repeated using 250 mL of ethanol (95%) as the extraction solvent, providing approx. 1000 mg of extract. The extract was then hydrodistilled and used in column chromatography. ***Fractination using Column Chromatography***: Column chromatography was performed on a Biotage, Inc. (Charlotte, NC) IsoleraTM pump equipped with an IsoleraTM flash collector and variable wavelength detector. The extract was fractionated using an XP-Sil, 100 g, SNAP cartridge (40–63 µm, 60 Å, 40 x 150 mm) running at 50 mL min-1 using a two solvent gradient from hexane to hexane:ethyl acetate (70:30) over 2400 mL while monitoring at 254 nm. Column eluate was collected into 22 mL portions and based on TLC similarities, recombined into 4 fractions (A, B, C, and D). This process was repeated twice using multiple batches of the extract from above.

Objective 9: Use the four fractions (from objective #8) for trials in the MSU captive deer facility and research fields to determine which fraction(s) is most effective in repelling deer and insect. Sicklepod extract was used as an insecticide comparing to neem oil and bifen, the results were as follows:

Figure 10. Leaf disc images after 48 hour-feeding with two loppers per cup with alternate food. The treatments from left to right are: A. control; B. neem oil; C. sicklepod extract; D. bifen.

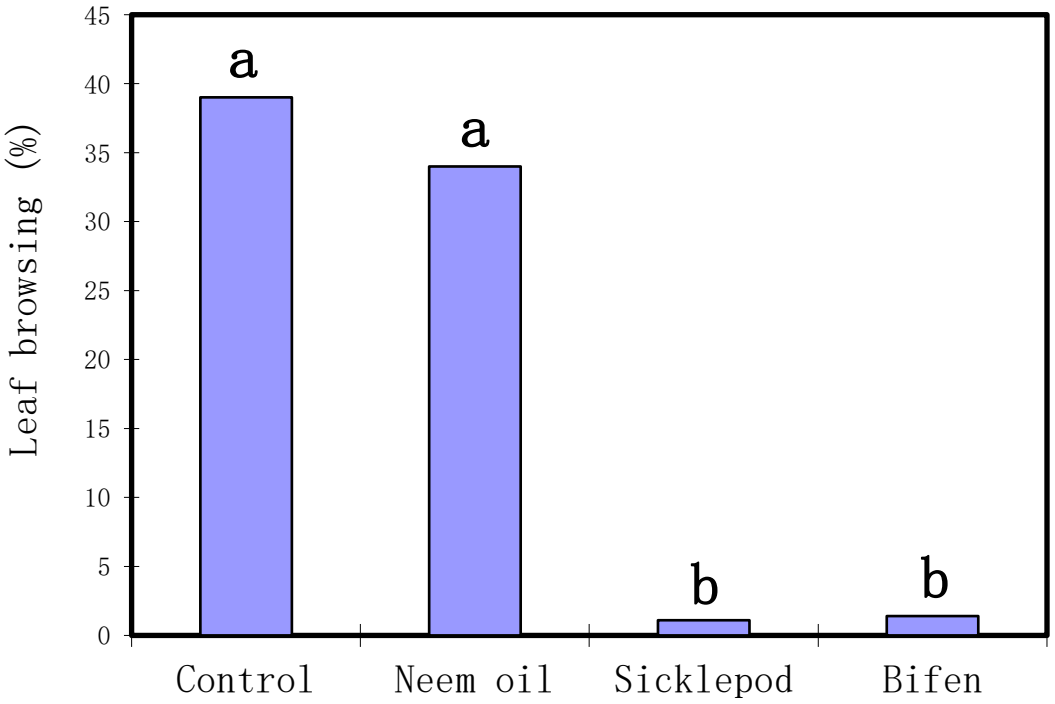


Figure 11**.** Leaf percentage fed after 48 hours. The statistical letters showed sicklepod extract and bifen had same antifeedant effect, while neem oil and control had same and higher fed percentage. Number of replicates=10.

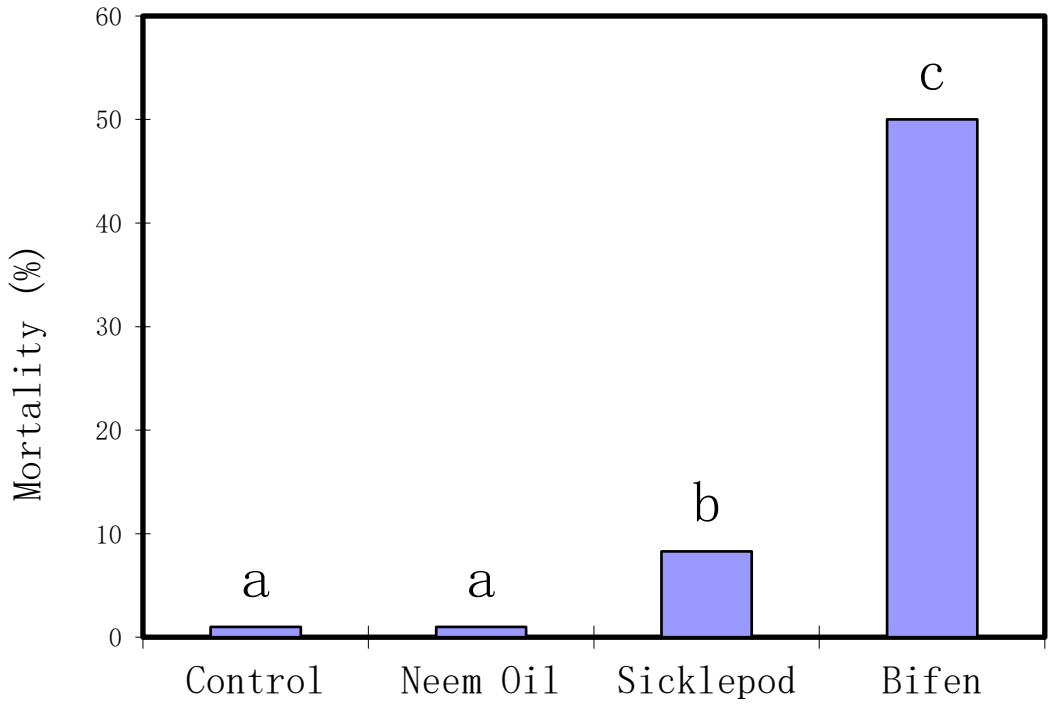


Figure 12. Mortality of different treatments in leaf disc experiments after 48 hours (n=3).

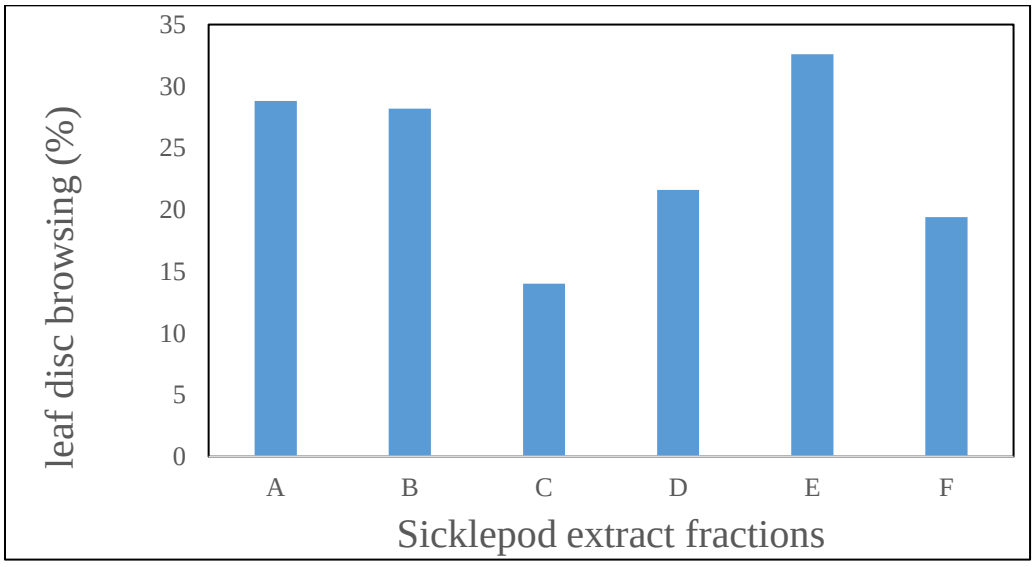


Figure 13. Tentative data of antifeedant effects of sicklepod extract fractions. Due to hydrophobicity (hydrophilicity) difference, fractions A, B, and C used water-based solvent while fractions D, E, and F used methanol-based solvent. Hence the comparison was not under same condition. In addition, the results were not statistically robust. To be repeated.

The original sicklepod extract was water-based extract which was rich in sugar, and anthraquinone concentration was limited by its high viscosity (150 ppm limit). The sicklepod-methanol extract was rich

in oil, which can be dispersed by surfactant. Our tentative reformulation reached 220 ppm anthraquinone derivatives resulting in a 47% increase in the anthraquinone concentration. Using the above new formulation, good insect antifeedant results were obtained (Figure 14). Field data using the above reformation is presented in Figure 15 & 16.

Figure 14. Row B was plain water extract (100 ppm); row A and row C were enriched by methanol and ethyl acetate extract respectively (200 ppm). The feeding experiment lasted two days.

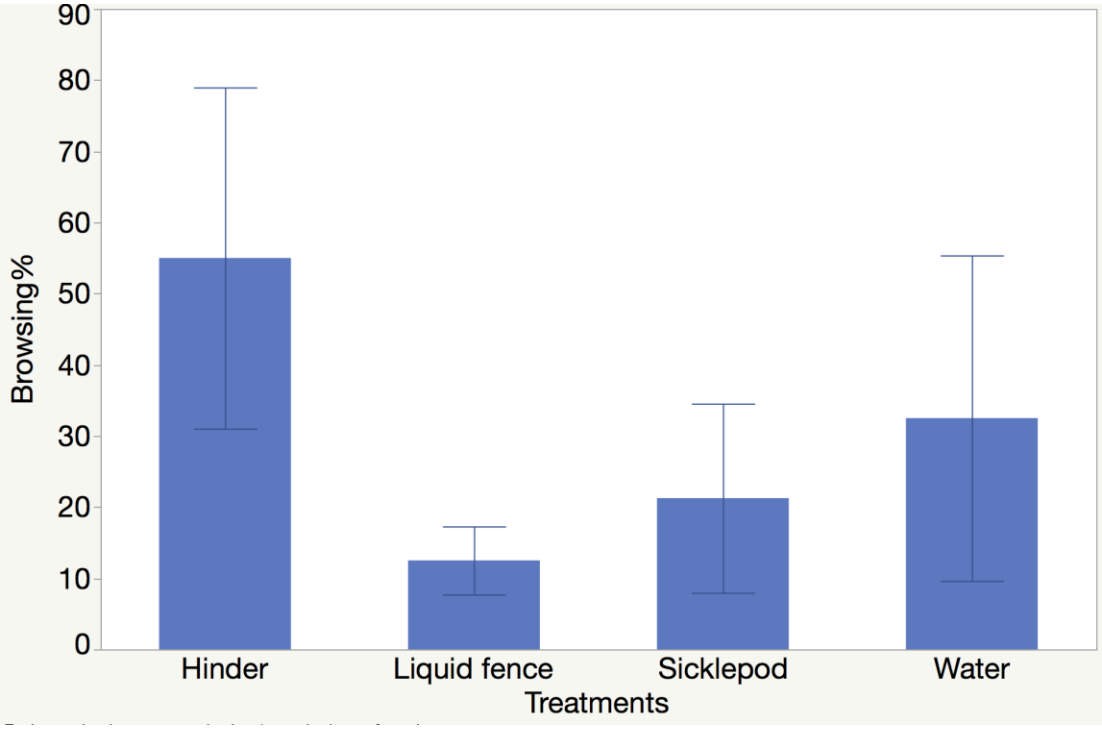


Figure 15. Three-day browsing data

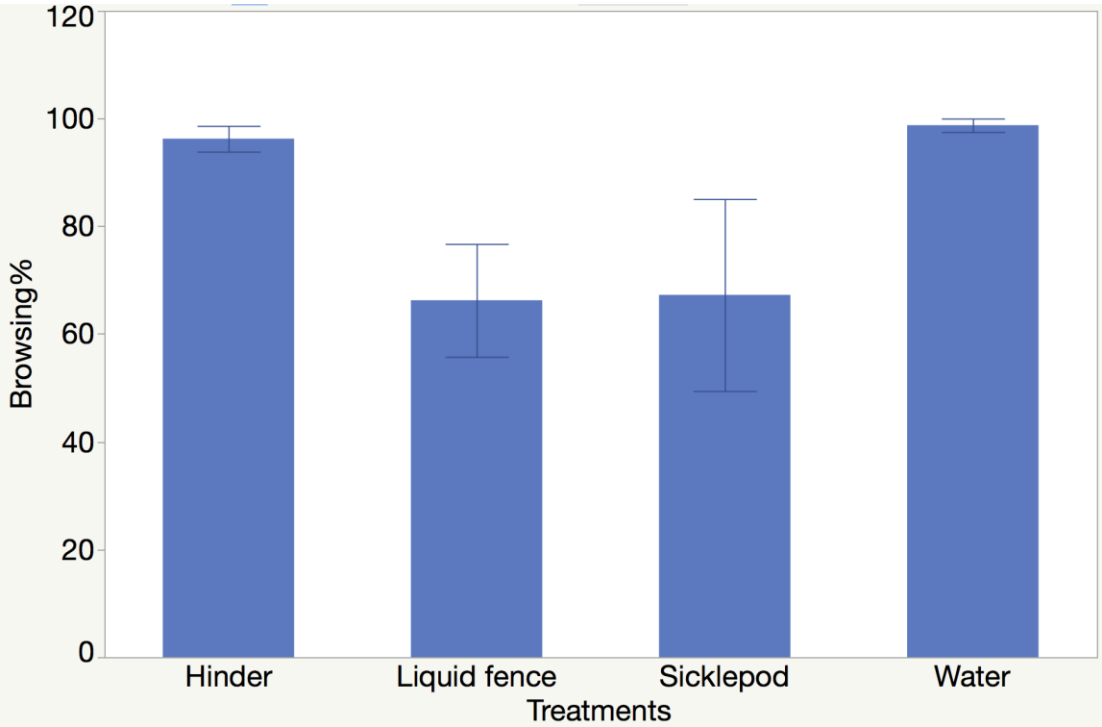


Figure 16. Eleven-day browsing: This is the first time sicklepod extract had same browsing rate as liquid fence. Maybe related with the methanol extract enrichment.

Objective 10: Identify specific compounds in fraction(s) (from objective #9) putatively associated with deer repelling property, using NMR spectroscopy, chromatography analyses. Fifty wild soybean accessions were screened for soybean looper resistance, three accessions proved to be resistant to soybean looper. Assumed active ingredients of sicklepod extract were anthraquinone derivatives, they showed characteristic yellow-orange fluorescence under UV A (Figure 17). The yellow fluorescence was observed in the three looper resistant wild soybean accessions (Figure 18).

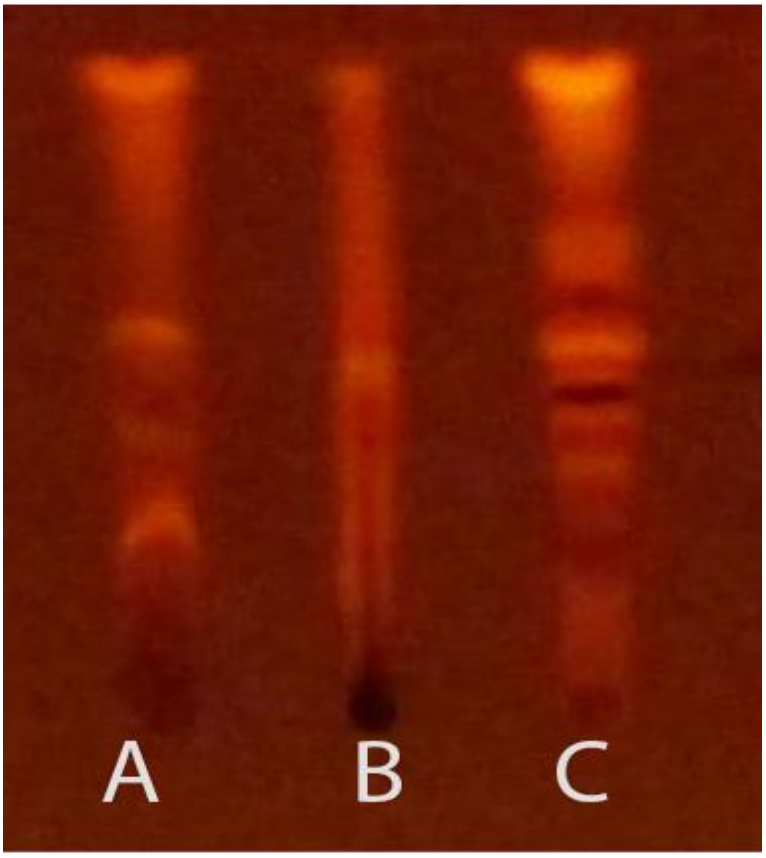
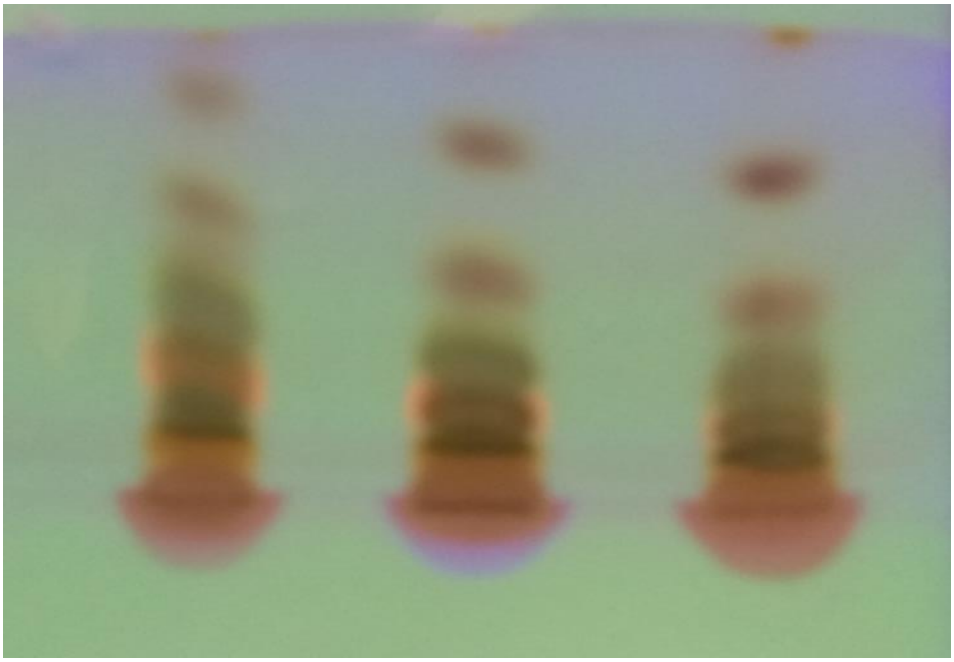


Figure 17. Yellow fluorescence from sicklepod extract indicated existence of anthraquinone derivatives.



WS22 WS5 WS3

Figure 18. Yellow fluorescence was identified in the three looper resistant wild soybean leaf extract, indicating existence of anthraquinone derivatives.

Tissue scale anthraquinone (yellow fluorescence) distribution imaging was conducted (Figure 19, 20), and similar images will be taken for the anthraquinone-containing wild soybean accessions. Additional 50 wild soybean accessions are growing in greenhouse for further screening for anthraquinone derivatives (insect resistance). In addition, sicklepod extract fractions A, B, C, D, E are ready for further insect test as previous data were not statistically robust. (This work was delayed due to sample problem)

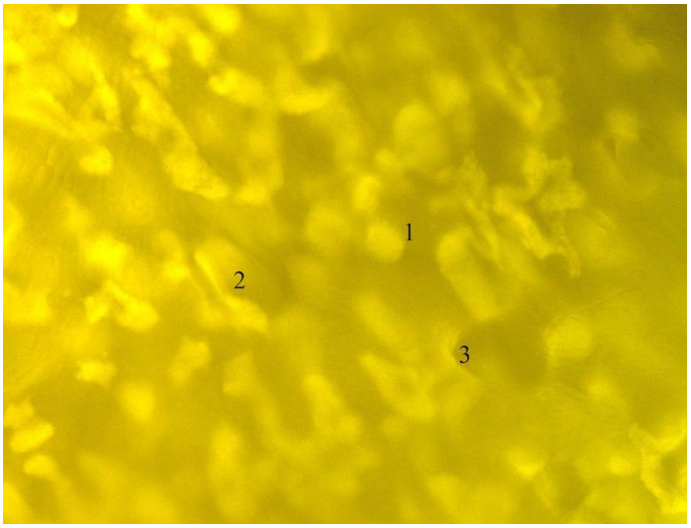


Figure 19. Anthraquinone distribution in sicklepod endosperm (yellow fluorescence).

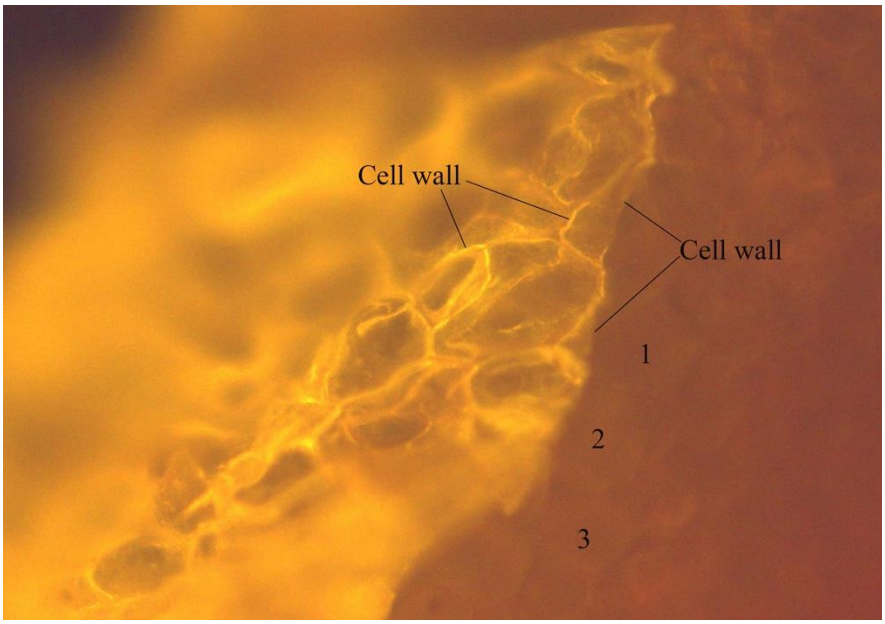


Figure 20. Anthraquinone distribution in sicklepod leaf (yellow fluorescence).

Impacts and Benefits to Mississippi Soybean Producers

The primary beneficiaries of the project will be all soybean growers in Mississippi, who represent over 2.3 million acres across the state. The estimated average yield for soybean in Mississippi is about 46 bushels per acre, and the soybean production in 2016 is estimated at 112 million bushels or $900 million in production value. Considering up to 26% and 41% yield reduction caused by deer and insect herbivory, respectively, the estimated economic loss could be $234 and $369 million annually in Mississippi. Developing a low cost sprayable is the first step in solving this problem. The field studies confirmed sicklepod as having anti-herbivore potential. The HPLC analysis identified anthraquinone derivaties and glycosides responsible for the anti-herbivore property, particularly emodin, chrysophanol, and physcion. These compounds identified to be responsible for anti-herbivore property will allow us to locate the biochemical pathway and genes related to the production of the particular compound. Molecular markers can then be developed associated with this pathway/genes, which is in turn linked to the anti-herbivore compound. Using these anti-herbivore markers, we can screen row crops, and vegetables for the anti-herbivore trait, or use it in molecular breeding to breed the anti-herbivore trait into crops. Crops with significant anti-herbivore property will prevent yield losses incurred due to herbivores such as deer. Soybean with significant anti-herbivore property will prevent yield losses incurred due to herbivores especially deer and insects.

The primary beneficiaries of the project will be all soybean growers in Mississippi, who represent over 2.3 million acres across the state. The estimated average yield for soybean in Mississippi is about 46 bushels per acre, and the soybean production in 2016 is estimated at 112 million bushels or $900 million in production value. Considering up to 26% and 41% yield reduction caused by deer and insect herbivory, respectively, the estimated economic loss could be $234 and $369 million annually in Mississippi. Developing a low cost sprayable is the first step in solving this problem. A second step is breeding soybean plants to reduce herbivory innately. Both of these approaches will be environmentally friendly and organic because they alleviate the need for pesticides.

Indirect benefits: The field testing confirmed candidate soybean accessions having anti-herbivore potential. The HPLC analysis identified the concentration of anti-herbivory compound responsible for the anti-herbivore property. Also, environmental sustainability of agriculture will increase dramatically with reductions in the need for pesticides.

Direct benefits: Soybean with significant anti-herbivore property will prevent yield losses incurred due to herbivores especially deer and insects.