MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 08-2018 (YEAR 2)
2018 ANNUAL REPORT

Title: Using weeds as a resource to develop herbivore-resistant soybean

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BACKGROUND AND OBJECTIVES

High density herbivore populations are problematic in agricultural areas because they can cause economic losses by damaging crops. Up to $68/ha or 43% financial losses from white-tailed deer herbivory have been reported. We observed the same trends on research soybean plots at our North Farm Research Facility with a relatively large area and a relatively low deer density where damaged areas had as much as 80% reduction in yield (**Figure 1**). Similarly, insect herbivory in some cases causes complete crop loss for producers.

Insecticides are expensive and often harmful to the environment. Among the current solutions to manage herbivores, fencing is expensive and labor intensive and requires weekly inspection to ensure effective operation, while repellents lose effectiveness after rainfall. With increasing costs of agricultural production, the economic impact from reduced yield is highly important, especially for smaller farms not financially buffered against poor harvest due to yield losses. It is therefore critical to protect soybean yield against herbivory.

Weeds, because of their vast genetic and phenotypic diversity, are a good resource for anti-herbivore traits. Studies by us (in Year 1) have shown that sicklepod weed seeds and plants contain high amounts of anthraquinone which may be the most effective chemical antiherbivory strategy of any plant on earth against both insects and mammals. In Year 1, we were able to demonstrate, through our captive deer and field studies, that anthraquinone extracts from sicklepod applied on soybean are 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. Using chromatography techniques, we also found up to 11 times higher amounts of anthraquinone in sicklepod than in soybean plants.

The objectives for Year 2 of this project are:
(1) Screen diverse soybean germplasm that consists primarily of wild accessions and some commonly grown cultivars in the southern US, for high anthraquinone production;
(2) 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
(3) Conduct quantitative trait loci (QTL) analysis to identify molecular markers associated with anthraquinone-related genes in soybean.

REPORT OF PROGRESS/ACTIVITY

Objective 1: 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 and 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, thus, they were 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 weighed 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: Sicklepod leaf powder of 1.0 g was weighed 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 a 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 2**), thus suggesting that there may be additional compounds associated with deer and/or insect repelling property of wild soybean. There is thus a need to identify additional unknown compounds other than anthraquinone that may be significantly associated with the deer and/or insect repelling property, and a combination of column chromatography and NMR spectroscopy will help achieve this.

Objective 2: 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 2**). 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 3**), thus suggesting wild soybean accessions to be as effective as sicklepod in repelling deer.

Objective 3: Conduct quantitative trait loci (QTL) analysis to identify molecular markers associated with anthraquinone related genes in soybean.

Leaf tissues from each soybean accession 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.

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 material 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 will confirm candidate soybean accessions having anti-herbivore potential. The HPLC analysis will identify the concentration of anti-herbivory compounds responsible for the anti-herbivore property. Also, environmental sustainability of agriculture will increase dramatically with reductions in the need for pesticides.

Direct benefits: The project will identify molecular markers associated with the pathway/genes for the anti-herbivory trait. These molecular markers can be used in molecular breeding to breed anti-herbivory traits into soybean. Soybean with a significant anti-herbivore property will prevent yield losses incurred due to herbivores especially deer and insects.

END PRODUCTS–COMPLETED OR FORTHCOMING

Publications

1. Yue, Z.M., **T. M. Tseng**, and M. Lashley (2018) Characterization and Deer-Repellent Property of Chrysophanol and Emodin from Sicklepod Weed. American Journal of Plant Sciences, 9, 266- 280. https://doi.org/10.4236/ajps.2018.92022
2. Tseng, T.M. (2018) From foes to friends: Exploiting the agricultural potential of weeds. Scientia Global https://doi.org/10.26320/SCIENTIA166
3. Yue, Z., and Tseng, T.M., 2018. Tissue distribution and subcellular localization of anthraquinone derivatives in sicklepod plants. (In preparation for submission to *Frontiers of Plant Science*).

Abstracts

1. Yue Z., and **T. M. Tseng** and M. Lashley (2019) Sicklepod Extract as an Effective Deer Repellent: From Captive Facility to Field Testing. In *Proceedings of Southern Weed Science Society*, vol. 72.

2. Yue Z., and **T. M. Tseng** (2019) Preparation of Sickepod Extract with Potential Use as an Effective Deer Repellent. In *Proceedings of Southern Weed Science Society*, vol. 72.
3. Yue, Z., M. Lashley, and **T. M. Tseng** (2018) Evaluation of sicklepod extract as a natural deer repellent for food crops and vegetables. In *Proceedings of ASA, CSSA and SSSA International Annual Meetings*, vol. 153.
4. Yue Z., and **T. M. Tseng** (2018) Study of anthraquinone biosynthesis, transport and storage in sicklepod weed using fluorescence imaging. In *Proceedings of Southern Weed Science Society*, vol. 71.
5. Yue Z., M. Lashley, S. Shrestha, G. Caputo, and **T. M. Tseng** (2018) Field testing of sicklepod extract as effective deer repellent to protect soybean. In *Proceedings of Southern Weed Science Society*, vol. 71.

Graphics/Tables

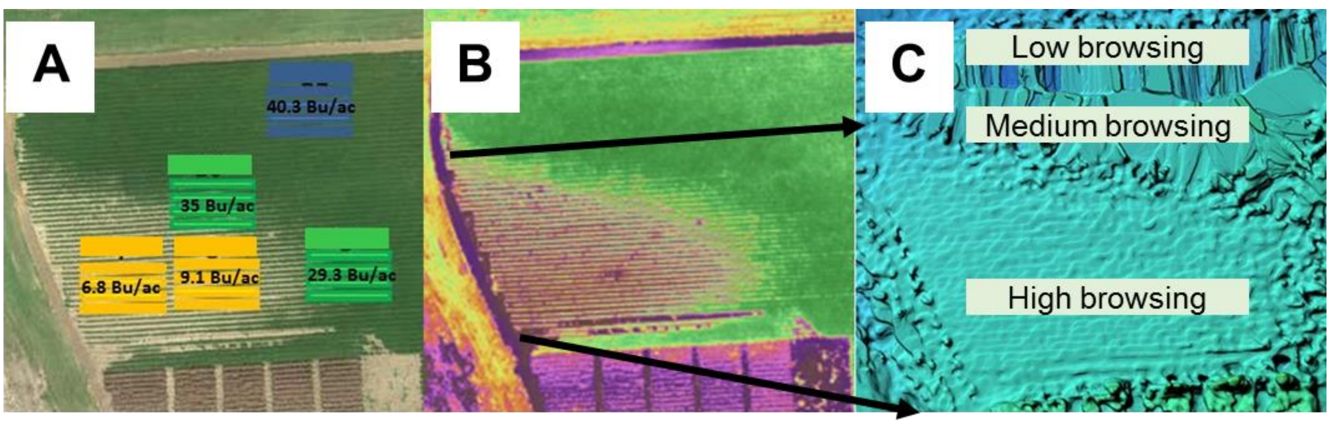

Figure 1. Yields (panel A), thermal imaging (panel B), and 3D structural signature (panel C) in soybeans following low (blue), medium (green), and high (light green) deer damage as measured with an unmanned aerial vehicle.

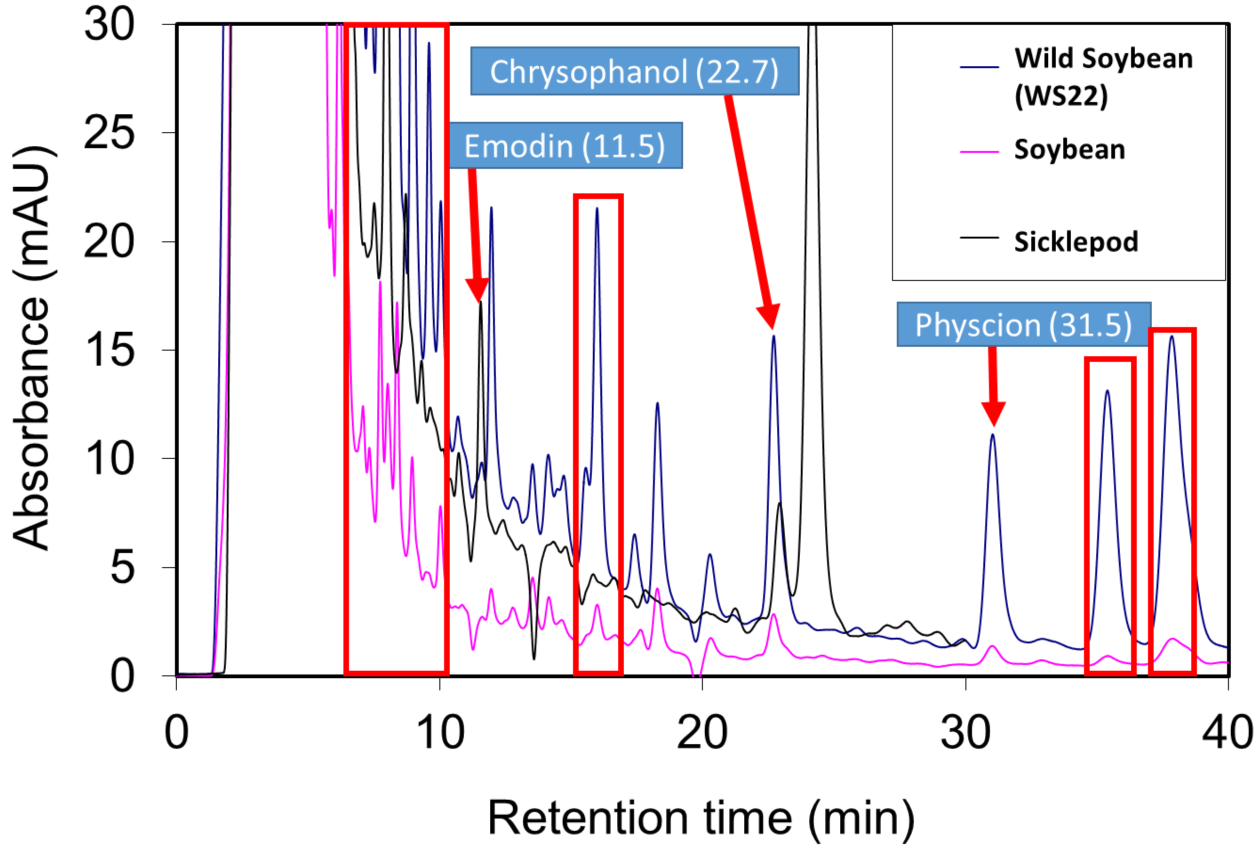

Figure 2: Stacked chromatogram of wild soybean (WS22), sicklepod, and soybean leaf extract showing anthraquinone contents (emodin, chrysaphanol, and physcion). Soybean showest the lowest anthraquinone content, while wild soybean contain equal or higher anthraquinone content than sicklepod. The chromatogram 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.

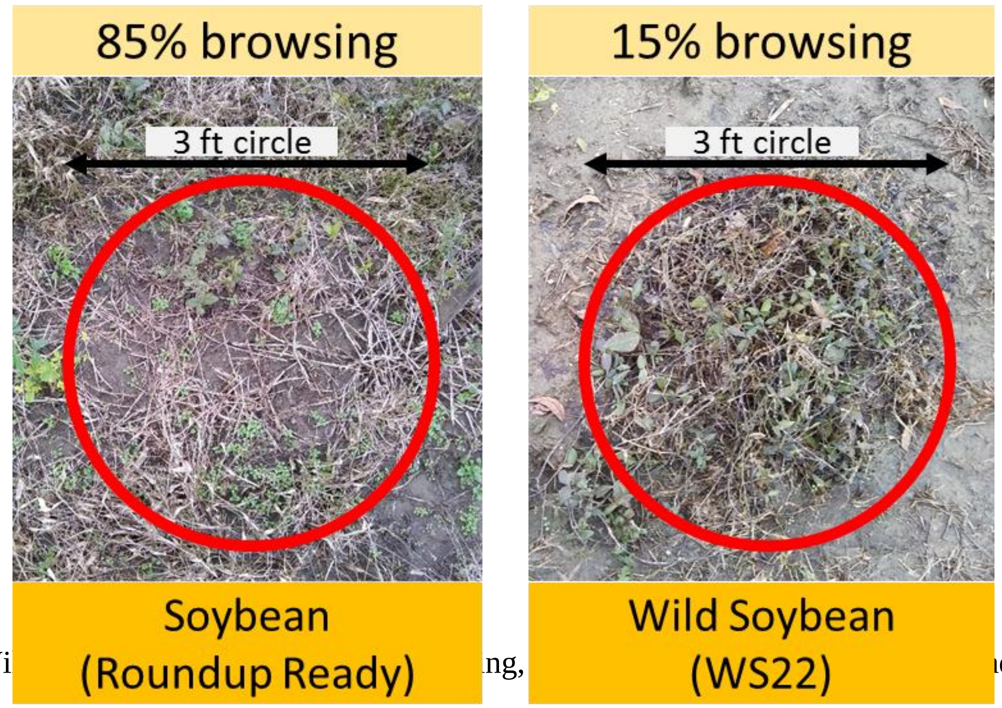

Figure 3: Wi ng, dy) with 95% browsing, thus suggesting wild soybean (WS22) to be effective in repelling deer.