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| Project Title | | Exploitation of weed species extracts as an effective and environmentally friendly strategy to control insects and deer in soybean | | | | | |
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| Additional PIs  For this project | | Natraj Krishnan, Associate Professor of Insect Physiology, Mississippi State University, 662-325-2978, nk260@msstate.edu. | | | | | |
| Research Locations (and states involved) | | Starkville, Mississippi. | | | | | |
| **Timeline:**  **Current Year - FY22** | | | **Multi-Year Project Information** (if applicable) | | | | |
| Year 1 | Year 2 | | | Year 3 |
| Start Date | 01/01/2022 | | **01/01/2022** | **01/01/2023** | | |  |
| End Date | 12/31/2023 | | **12/31/2022** | **12/31/2023** | | |  |
| Funds Requested | $80,813 | | $38,907 | $41,906 | | | $ |
| **Program Area (e.g., breeding, mngt.): Insect and Deer Management/Control** | | | | | | | |
| Objectives | | The objectives of the project will be to (1) prepare plant extracts of potential weed species (sicklepod, coffee senna, hemp sesbania, and prickly sida) 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. | | | | | |
| Justification | | High-density herbivore populations are problematic in agricultural areas as 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 our soybean research plots with a relatively large area and low deer density, where damaged areas had as much as 80% yield reduction (Fig. 1). Similarly, insect herbivory can cause complete crop loss for producers. Insecticides are expensive and often harmful to the environment. Among current solutions to manage herbivores, fencing is costly and labor-intensive, requiring weekly inspection to ensure effective operation, while repellents lose effectiveness after rainfall. With increasing agricultural production costs, the economic impact from reduced yield is increasingly essential, especially for smaller farms not financially buffered against the poor harvest. It is, therefore, critical to protect soybean yield against herbivory. Weeds, because of their vast genetic and phenotypic diversity, are an excellent resource for anti-herbivore traits. | | | | | |
| Exp Setup | | 1. **Prepare plant extracts of potential weed species and develop liquid formulations for application on soybean crop**: Plant extracts of four potential weeds (sicklepod, coffee senna, hemp sesbania, and prickly sida) will be prepared using four different methods. (1) Berries/fruits: Fruits will be homogenized followed by centrifugation at 3000g for 15 min; (2) Leaves and stem: Tissue will be homogenized, dissolved in 80% methanol, and centrifuged at 1500g for 10 min; (3) Leaves and stem: Tissue will be homogenized, dissolved in water, and centrifuged at 1500g for 10 min; and (4) Leaves and stem: Tissue will be homogenized, dissolved in 70% aqueous acetone, and centrifuged at 1500g for 10 min. Supernatant from each extraction will be collected, dissolved in 80% methanol, and freeze-dried. 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**: All powdered extracts will be dissolved in water to produce 10 different concentrations (0, 1, 10, 50, 100, 200, 400, 600, 800, and 1000 mg/mL). Liquid extracts from each of the four methods will be applied on soybean plants propagated in a field nursery at V4 (8-inch) stage. We will conduct field trials at Andrew’s Forestry and Wildlife Experiment station with high deer density and have paired 0.25 ha treated and control plots replicated three times. Deer and insect herbivory damage along line transect will be quantified once monthly for the duration of the life cycle of soybeans, and the use of each plot by all vertebrate species will be monitored continuously with trail cameras set to take photographs every minute. We will also conduct three UAV flyovers which will allow us to quantify three-dimensional structural damage resulting from herbivory in treatments and controls (**Figure 1**). 3. **Conduct HPLC and GC/MS analysis to identify target anti-herbivore compounds**: Fractions containing the anti-herbivore compound(s) will be collected using HPLC, followed by identifying the target compound(s) using GC/MS. Plant extracts will be loaded onto a C18 column, and the phenolic compounds will be eluted by a gradient of solvent B (82% (v/v) acetonitrile, 0.04% (v/v) phosphoric acid) ranging from 0-15%. Peaks will be detected at 280 nm and identified by comparing with retention times of standards. 4. **Conduct QTL analysis to determine molecular markers associated with the anti-herbivory trait in weed species**: A total of 30 simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers will be used to screen each of the weed species. DNA will be extracted from leaves and subjected to polymerase chain reaction to amplify the genetic regions associated with the target anti-herbivore compound(s) identified from objective 3. Markers showing significant linkage with anti-herbivory traits will be summarized. These potential markers will be used in future breeding experiments in the greenhouse to select soybean lines with the anti-herbivory trait(s). | | | | | |
| Summary | | High-density herbivore populations are problematic in agricultural areas as 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 our soybean research plots with a relatively large area and low deer density, where damaged areas had as much as 80% yield reduction (**Fig. 1**). Similarly, insect herbivory can cause complete crop loss for producers. Insecticides are expensive and often harmful to the environment. Among current solutions to manage herbivores, fencing is costly and labor-intensive, requiring weekly inspection to ensure effective operation, while repellents lose effectiveness after rainfall. With increasing agricultural production costs, the economic impact from reduced yield is increasingly essential, especially for smaller farms not financially buffered against the poor harvest. It is, therefore, critical to protect soybean yield against herbivory. Weeds, because of their vast genetic and phenotypic diversity, are an excellent resource for anti-herbivore traits. Preliminary studies have shown that sicklepod weed contains high amounts of anthraquinone, which may be the most effective chemical antiherbivory strategy of any plant on earth against insects and mammals. In our trials, soybean plants not applied with sicklepod extract were consumed completely, while plants applied with sicklepod extract were entirely avoided (**Fig. 2**). Moreover, sicklepod extract had no adverse effects on overall soybean yield (**Fig. 3**). We prepared and characterized four sicklepod fractions (A, B, C, D, and E) with improved deer and insect repelling efficacy. Leaf disc assays showed that fractions D and C resulted in an 80 and 52% reduction in soybean looper feeding (**Fig. 5**). Insects fed with soybean leaves treated with fractions D and C were also the smallest in size (**Fig. 5**). | | | | | |
| Key Metrics | | The proposed project will be managed following an established timeline to track progress in different areas of research. Built into this timeline is also the plan for an annual project meeting with all PIs and the Graduate Student. Following are the key performance indicators or project metrics:   1. We weed species having anti-herbivore potential: We anticipate confirming at least three of the four weed species to have deer repellent and insecticidal properties. 2. Compounds responsible for the anti-herbivore property: We anticipate identifying at least three anthraquinone compounds associated with anti-herbivore properties. 3. Molecular markers can be developed associated with the pathway/genes for the anti-herbivory trait: We anticipate identifying at least five molecular markers highly associated with the anti-herbivory trait. | | | | | |
| Expected Deliverables | | Project outcomes from each year will be shared as PowerPoint and Poster presentations with researchers within the weed science community at The American Society of Agronomy, Crop Science Society of America (ASA-CSSA-SSSA) annual meeting, and the Entomological Society of America. Also, results of our findings will be presented at the Producer Advisory Meeting at Verona, MS, to share outcomes of the field screening, and highlight the efficacy of different weed species extracts applied on soybean plants in repelling herbivory and insects. We expect to publish at least 2 papers, open access, in high-impact journals such as Pest Management Science, Annals of Botany, Weed Research, or Scientific Reports. | | | | | |
| Benefit to midsouth farmers | | 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, totaling more than $900 million in production value. Considering an up to 26% and 41% yield reduction caused by deer and insect herbivory, respectively, based on literature, the estimated economic loss can be up to $234 and $369 million annually in Mississippi, from deer and insects, respectively. Breeding soybean plants to reduce herbivory innately would alleviate the need for pesticides and labor, further reducing producer costs. It is therefore critical that we take necessary steps to prevent herbivory on soybean fields.  Indirect benefits: The field testing of plant extract from different weed species will help shortlist candidate weed species having anti-herbivore potential. The HPLC analysis will identify the target compound responsible for the anti-herbivore property. Identifying the compound(s) responsible for anti-herbivore property will allow us to locate the biochemical pathway and genes related to the production of the particular compound(s). Also, the environmental sustainability of agriculture will increase dramatically with reductions in the need for pesticides and other synthetic chemicals.  Direct benefits: Molecular markers can be developed associated with the pathway/genes for the anti-herbivory trait. Using these molecular markers, we can screen soybean germplasm for the anti-herbivore trait or use it in molecular breeding to breed these traits into soybean (since sicklepod, coffee senna, and hemp sesbenia are close relatives of soybean). Soybean with significant anti-herbivore properties will prevent yield losses incurred due to herbivores, especially deer and insects. | | | | | |
| Progress Made | |  | | | | | |
| Signature of Principle Investigator | | | | | | Date: | |
| A picture containing insect  Description automatically generated | | | | | | 07/29/2021 | |