51-2020, Sicklepod extract formulations as natural and effective deer and insect repellent

Annual 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 (previous MSPB 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 is the establishment of fences or application of repellents (Cauteren et al. 2006). Fencing is expensive and labor-intensive to install and requires weekly inspection and maintenance throughout the growing season to ensure effective operation and longevity (Ward et al. 2010). The effectiveness of repellents is also reduced by rainfall that may dissolve repellents requiring reapplication and are ineffective against deer. Moreover, soybean insect pests, especially soybean aphids and loopers, 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.

Plants possess varying levels of herbivore defense mechanisms, and weeds, because of their vast genetic and phenotypic diversity, are excellent resources 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 our previously funded MSPB project, we conducted tests at the 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 applying soybean plants in a diet selection trial on captive deer and insects. Soybean plants not applied with sicklepod extract were consumed completely, while plants with sicklepod extract were entirely avoided. Moreover, the sicklepod extract had no adverse effect on the overall soybean yield. Insect damage was apparent in our treatment area. Our data suggest that soybean plants treated with sicklepod extract had lesser insect damage than two other insect repellants available in the market. Our preliminary leaf disc insect feeding results indicate that sicklepod extract and Bifen (synthetic insecticide) had similar antifeedant effects, with both exhibiting only 2% feeding. In comparison, neem oil and control showed 34 and 38% feeding, respectively. The antifeedant effect of sicklepod extract and Bifen were similar, while the control and neem oil treatments had lower and similar antifeedant effects. 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.

The limit of our current sicklepod extract formulation is the high viscosity, which prevents the formulation of an extract with a high anthraquinone concentration (ideally >300 ppm). Increasing the anthraquinone concentration of the extract results in increased viscosity of the spray fluid, making it hard to apply through spray booms. Sicklepod seeds contain large amounts of galactomannans, a polysaccharide often used to increase viscosity of solutions, primarily upon heating. There is, therefore, a critical need to increase the efficacy of the sicklepod extract by increasing its active ingredient concentration (anthraquinone concentration to >300 ppm; at present only <100 ppm) and decrease the viscosity (not to clog spray). Our preliminary experiments show sicklepod seeds extracted in methanol (20%) or ethyl acetate (10%) resulted in high anthraquinone concentrations (>200 ppm), resulting in an effective leaf antifeedant effect against soybean loopers. In addition, a nonionic surfactant can be used to increase the spreading of the extract on the soybean leaf surface to provide long-lasting insecticidal and deer-repelling effects. The use of organic solvents such as methanol, chloroform, acetone for extracting anthraquinone in sicklepod will allow the removal of the galactomannans, eventually increasing the concentration of anthraquinone >300 ppm in the extract.

OBJECTIVES:

(1) prepare and characterize four formulations of sicklepod extract for improved deer and insect repelling efficacy;
(2) use the four formulations (from objective #1) for trials in the captive deer facility, and leaf-disc assay to determine which formulation(s) is most effective in repelling deer and insect, respectively; and,
(3) select effective formulations (from objective #2) for trials in forestry and agronomy research fields using unmanned aerial vehicles (UAVs), plant surveys, and trail cameras to quantify deer and insect use and damage to soybeans in treatment and control plantings.

REPORT OF PROGRESS/ACTIVITY:

- In Year 1, 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 80 and 52% reduction in soybean looper feeding (**Fig. 1**). Insects fed with soybean leaves treated with fractions D and C were also the smallest in size (**Fig. 1**). Additionally, in our chromatography (HPLC) analysis, fractions D and C showed the highest anthraquinone concentrations (15 and 8 ppm, respectively), thus confirming the insecticidal property of anthraquinone.
- Soybean field trials conducted in Year 1 showed that our sicklepod formulation was most effective in repelling deer; better than Hinder (commercial deer repellent) and control (water) (**Fig. 2**).

  Our observations indicate deer is the dominant pest to soybean production in MS in June, while insects become the dominant pests during July, Aug, and Sep. Comparing to July, Aug, and Sep, when deer browsing of soybean focuses on plot edges, the deer browsing leads to soybean defoliation and lowering of soybean height, deer browsing in June extends far to the center part of soybean plots. The browsing results can be represented in two categories: (A) browsing above cotyledons and (B) browsing below cotyledons (**Fig. 4**). Soybean plants in category (A) can still branch from cotyledon and survive, while soybean plants in category (B) will die, leading to seedling loss. Such almost randomly distributed patches of seedling loss usually cannot be supplemented by replanting, leading to yield loss. Deer browsing of soybean starts as soon as soybean emerge. As soybean plants are lower in height than deer in June, deer view is not blocked, so deer activity (browsing) can reach all the soybean fields from edge to center. In July, soybean plants grow as high as deer, blocking deer view, and deer browsing is confined to the edges of the soybean field, which we usually see.

  In contrast to our previous deer repellent experiments in 2020, we sprayed our deer repellents at the V2 stage rather than the V5 stage at the optimum planting date (late May) in 2021 (**Fig. 5**). From the lessons learned from our previous deer repellent experiments in the agricultural field (in 2020), we limited our number of treatments to only three: sicklepod extract, sicklepod extract with glyphosate, and DeerPro (commercial). The repellents were sprayed on June 5, 2021, and the study is ongoing.
- The limit of our current sicklepod extract formulation is the high viscosity, which prevents the formulation of an extract with high anthraquinone concentration (ideally >300 ppm)—increasing the anthraquinone concentration of the extract results in increased viscosity of the spray fluid, making it hard to apply through spray booms. Sicklepod seeds contain large amounts of galactomannans, a polysaccharide often used to increase the viscosity of solutions, primarily upon heating. There is, therefore, a critical need to increase the efficacy of the sicklepod extract by increasing its active ingredient concentration (anthraquinone concentration to >300 ppm; at present only <100 ppm) and decrease the viscosity (not to clog spray).
  - Experiments conducted in Year 1 show that sicklepod seeds extracted in methanol (20%) or ethyl acetate (10%) resulted in high anthraquinone concentrations (>200 ppm), and leading to effective leaf antifeedant effect against soybean loopers (**Fig. 3**).

IMPACTS AND BENEFITS TO MISSISSIPPI SOYBEAN PRODUCERS

The captive facility, greenhouse, and field screening identified and confirmed candidate sicklepod extract formulations with the most effective anti-herbivore (deer and insect) potential. The formulations were more effective than the commercially effective products, Hinder (deer repellent) and Bifen (insecticide). The availability of natural and effective anti-herbivory compound formulations identified in this study will lead to an increase in the environmental sustainability of agriculture with reductions in the need for synthetic pesticides.

END PRODUCTS – COMPLETED OR FORTHCOMING

Publications (2020-2021)

1. Yue Z., C. L. Cantrell, N. Krishnan, D. J. Lang, M. Shankle, T. M. Tseng (2021) Characterization of sicklepod extract as a deer repellent and insecticide for soybean looper (Lepidoptera: Noctuidae). In *Proceedings of Weed Science Society of America*, vol. 60.
2. Yue Z., I. S. Werle, S. Meyers, M. Shankle, T. M. Tseng (2021) Response of sweetpotato cultivars to dicamba and 2,4-D. In *Proceedings of Southern Weed Science Society*, vol. 74.
3. Yue Z., T. M. Tseng, and N. Krishnan (2020) Antifeedant effect of sicklepod extract on soybean looper *Chrysodeixis includens* (Lepidoptera: Noctuidae). *Mississippi Academy of Sciences Journal* 65(3):293-300.
4. Yue Z., T. M. Tseng, D. Lang (2020) Sicklepod Extract Preparation, Analysis, and its Antiherbivore Applications. In *Proceedings of Southern Weed Science Society*, vol. 73, p. 84.
5. Yue Z., N. Krishnan, T. M. Tseng (2020) Antifeedant Effect of Sicklepod (Senna obtusifolia) Extract on Soybean Looper *Chrysodeixis includens* (Lepidoptera: Noctuidae). In *Proceedings of Southern Weed Science Society*, vol. 73, p. 214.

Articles/Newspaper Columns

1. DAFVM LANDMARKS Magazine. (March 2019). Playing Defense - Animal Repellent Could Help Block Soybean Pests. http://www.dafvm.msstate.edu/landmarks/19/MarchLandmarks2019.pdf
2. MAFES DISCOVERS Magazine. (December 2017). Let go off my legume - Protecting soybeans through natural pesticide and precision ag. http://mafes.msstate.edu/discovers/article.asp?id=108

GRAPHICS/TABLES

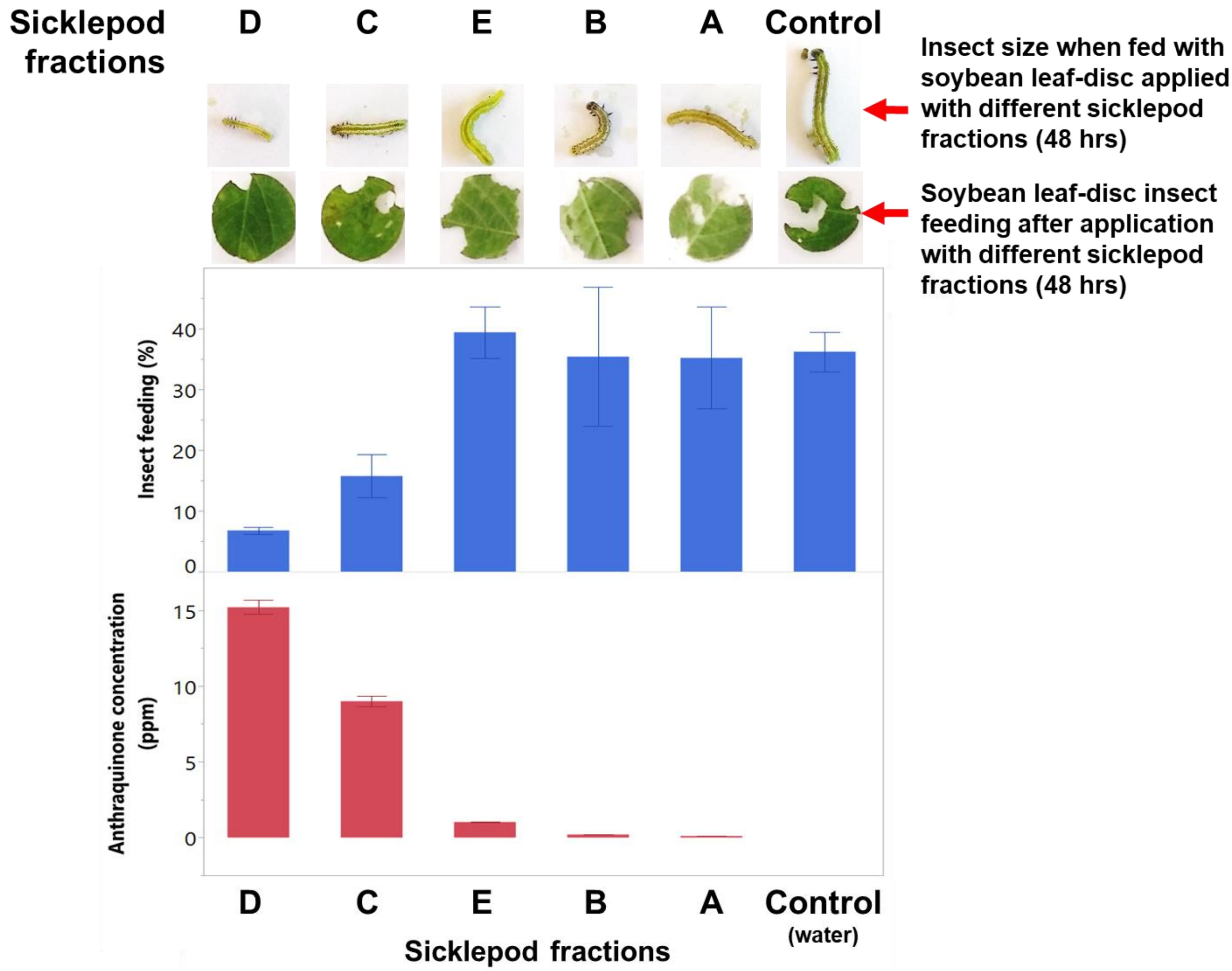

Figure 1. Anthraquinone content of different sicklepod fractions (A, B, C, D, and E) and its effect on insect feeding and insect size.

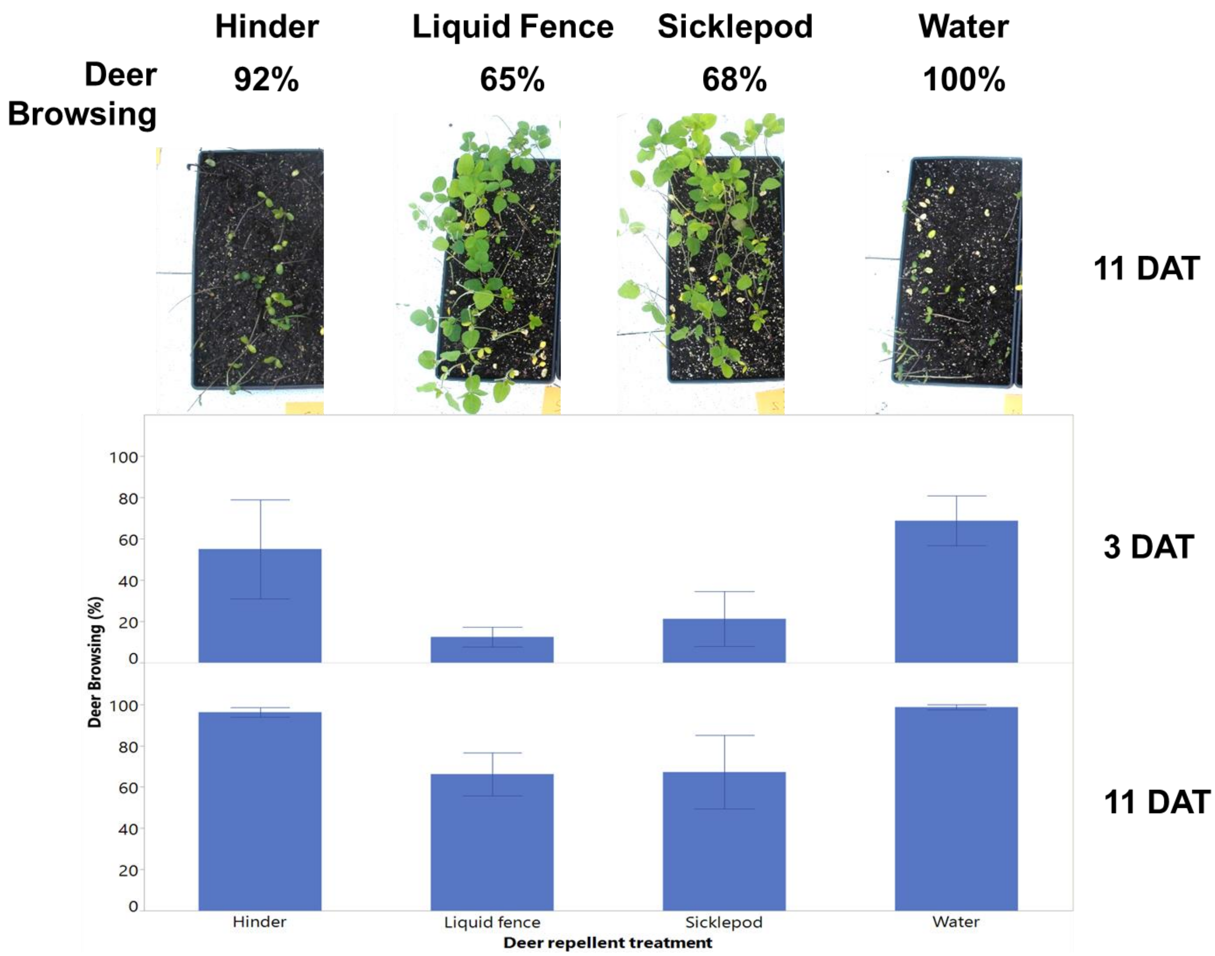

Figure 2. Soybean field trials showing deer browsing efficacy of sicklepod formulation compared to commercial deer (Hinder) and rabbit (Liquid Fence) repellents, and control (water).

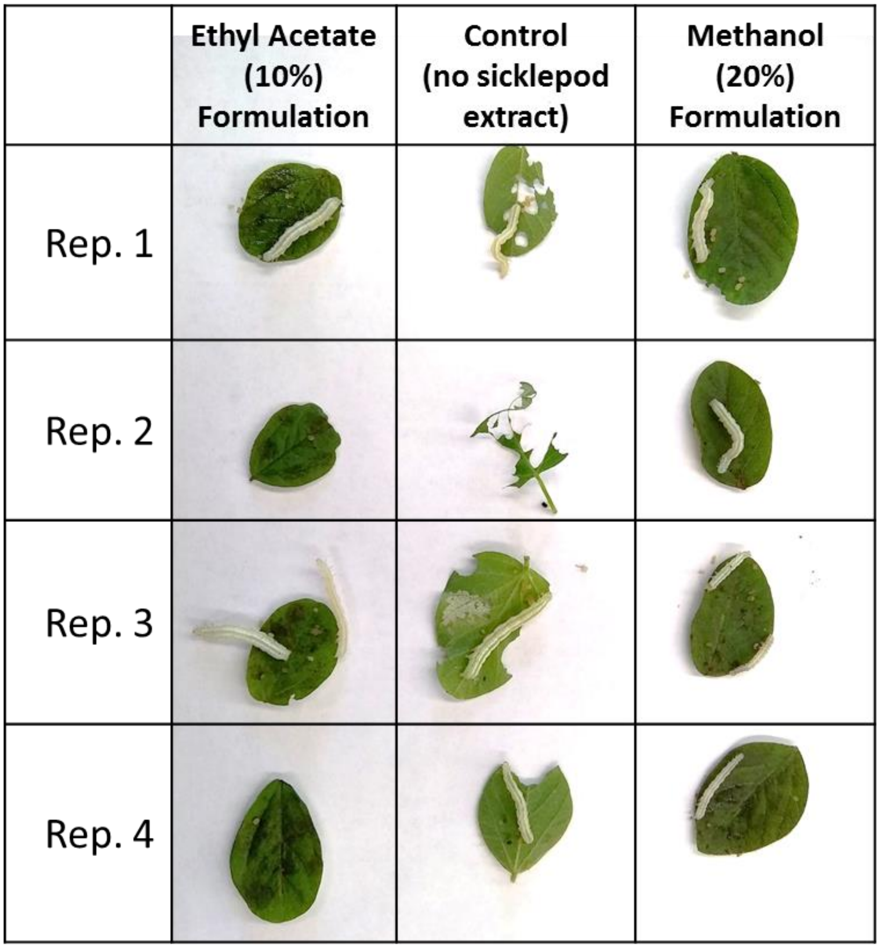

Figure 3. Soybean whole leaf assay to test different sicklepod extract formulations on soybean looper feeding. Both ethyl acetate and methanol formulations were effective in protecting soybean leaves from soybean looper feeding, compared to the control treatment where water was applied instead of sicklepod extract. The experiment was replicated four times and repeated twice. Two soybean loopers were added to each leaf. Not all insects were placed on the leaf when taking the above photo.

(A) browsing above cotyledon. (B) browsing below cotyledon

Figure 4. Soybean seedlings browsed by deer above (A) and below (B) the cotyledon leaves.

Figure 5. Field plot in 2021 showing a sicklepod treated row of soybean.