Final Project Report

Project title: Hyperspectral Imaging for Early Detection of Herbicide-Resistant Weeds in Soybean

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Specific Objectives:

1. Develop baseline spectral signatures of herbicide-resistant and susceptible weed bio-types
2. Use this information to detect herbicide-resistant weed biotypes in soybean fields using UAV-based hyperspectral imaging and machine learning algorithms
3. Develop herbicide-resistant weed maps in soybean fields in-season and at-harvest

Background

Waterhemp (*Amaranthus tuberculatus* [Moq.] JD Sauer) is one of the most troublesome weeds in soybean-based cropping systems of Iowa. Waterhemp populations resistant to seven different herbicide groups have recently been reported in the Midwest. Our recent survey indicate that 4-5 way resistance has evolved in waterhemp populations from Iowa. Early detection of resistant individuals in the field is key to developing effective weed management programs and mitigating herbicide resistance. Typically, herbicide-resistant weed biotypes are identified using whole-plant dose-response assays, target enzyme bioassays, and molecular/genetic markers specific to the resistance trait. However, these methods are laborious and time consuming. Hyperspectral imaging technology and machine learning algorithms can identify herbicide-resistant individuals from susceptible ones based on differences in reflectance patterns near UV, visible, and near IR region of the spectrum. This technology has proven successful in identifying biotic and abiotic plant stresses under field conditions. *The ultimate goal of this collaborative project is to accurately map (UAV-based) the location of herbicide-resistant weed biotypes in a production field using advanced optics and computer algorithms.*

Project Report:

This research is a collaborative effort between weed science experts at Iowa State University and remote sensing experts at Montana State University (MSU). All experiments and imaging were conducted at ISU. Data processing and machine-learning algorithms were developed at MSU.

Experiments under artificial lighting:

Greenhouse and laboratory experiments were started in late fall 2020 to identify spectral reflectance of different biotypes of waterhemp plants resistant to ALS inhibitors, atrazine, and/or glyphosate. Seeds used in these experiments were collected in fall 2019 for the survey of herbicide-resistant waterhemp populations in Iowa. Waterhemp seeds were planted in 25 cm × 51 cm × 5 cm flats containing Sunshine Mix #1/LC1 potting soil (Sun Gro Horticulture, Agawam, MA, USA) with sand (4:1 ratio) in the ISU Agronomy Greenhouse. Individual waterhemp seedlings were then transplanted into long-narrow plastic cones. Transplanted seedlings were fertilized and watered regularly. Eight plants (two from each biotype), with a height of 7-8 cm and similar leaf numbers were selected for the hyperspectral imagery (Figure 1). With the remaining seedlings, a standard whole-plant dose-response bioassay was conducted to confirm the sensitivity of these biotypes for three herbicides, imazethapyr, atrazine, and glyphosate.

Figure 1. Waterhemp plants (herbicide resistant and susceptible) currently grown in the ISU Agronomy greenhouse for hyperspectral imaging.

For the hyperspectral imaging, all measurements were taken at the ISU Weed Science laboratory using artificial light (provided by three 52-watt incandescent bulbs). The camera was mounted ~30 cm above the plant canopy. A Spectralon panel was placed at the same height as the plant canopy for reflectance calibration to compensate for the spectral variation due to the light source. Images were taken using a Pika L camera equipped with VNIR-17 mm lens (Resonon Inc., Bozeman, MT). Imaging was initiated by recording dark images with lens cap to subtract electronic noise from the raw image. Subsequent images were taken on selected waterhemp plants from each biotype, with Spectralon panel. When waterhemp reached a height of 10 cm, imaging was conducted utilizing a Pika L Hyperspectral Imager under artificial lighting.

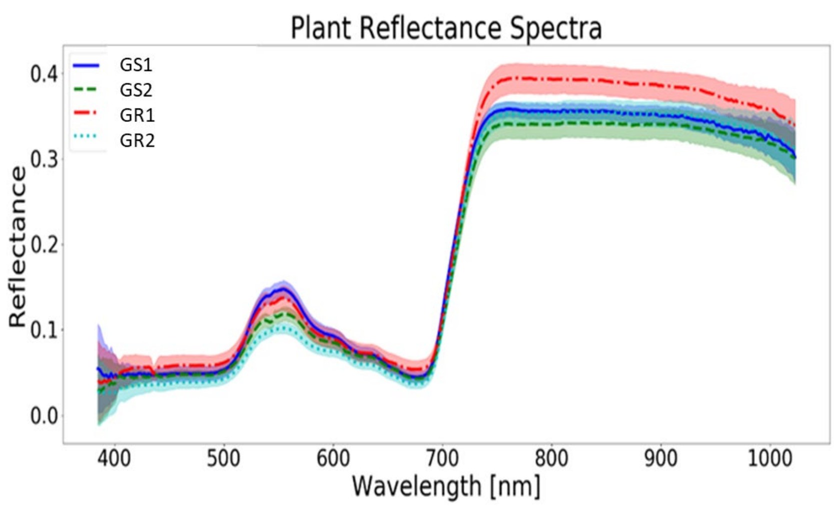

Figure 2. Mean (dotted lines) and standard deviation (shaded area) of spectral reflectance of different waterhemp biotypes [glyphosate-susceptible (GS) and glyphosate-resistant (GR)] showing separation at the NIR region.

This allowed us to obtain reflectance spectra for different biotypes. A Pika L software (SpectrononPro) was used to process the images. Results obtained from the controlled experiments are summarized in Figure 2. The reflectance spectra represent the average and standard deviation of spectra obtained from each plant. The curves represent spectral reflectance of different waterhemp biotypes. As evident in Figure 2, significant spectral differences between biotypes would require a more sophisticated analysis to develop accurate classification systems.

Field experiments in Soybean (2021):

Field experiments were conducted in June of 2021 in soybean fields near Ames, IA. The Pika L Imager was mounted onto a DJI M600Pro drone. Drone flights occurred over soybean fields with known herbicide-resistant waterhemp populations. Spectral data were extracted from the Pika L imager using a Resonon software *Spectronon Pro* to develop classification images using a neural network (machine learning algorithm). A PCA plus logistic regression code was used in *Python* to analyze the spectral data.

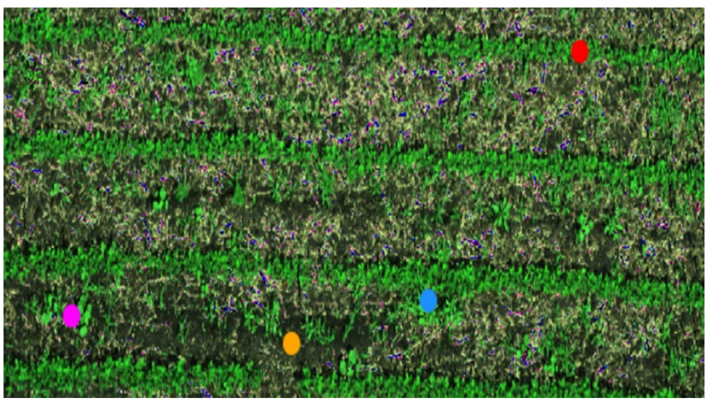

Figure 3. Hyperspectral image recorded by Pika L Imager mounted to a drone in a soybean field.

Figure 4: Demonstration at the ISU weed science field day, July 2021.

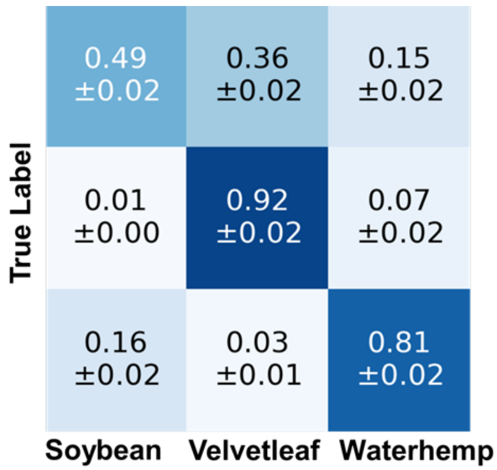

Figure 5: Confusion matrix of machine learning for soybean, velvetleaf, and waterhemp showing accuracy of detection.

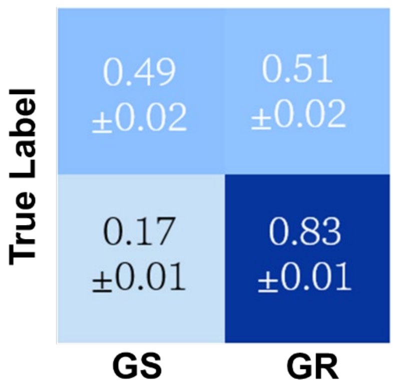

Figure 6: Confusion matrix of machine learning for glyphosate-resistant (GR) and susceptible (GS) waterhemp biotypes showing accuracy of detection.

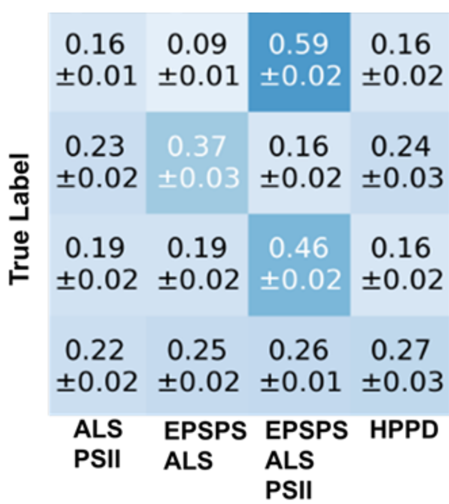

Figure 7: Confusion matrix of machine learning for herbicide-resistant waterhemp biotypes showing accuracy of detection.

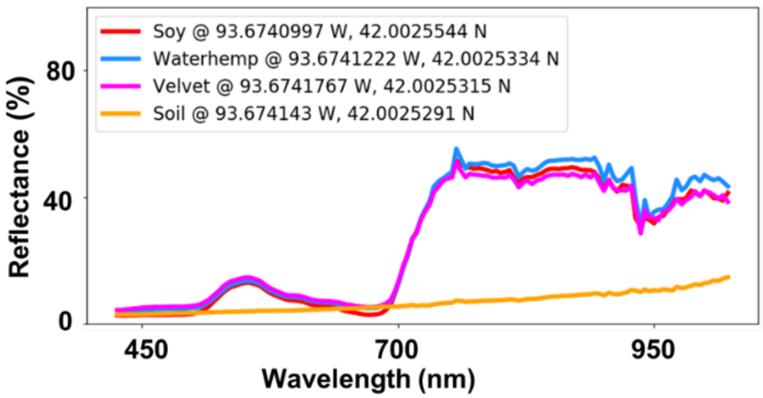

Figure 8: Spectral reflectance of waterhemp, velvetleaf, and soybean.

Weeds were differentiated from soybean with >95% accuracy in the field (Figures 5 and 8). Waterhemp was differentiated from other weedy species with 81% accuracy. One-way resistance to glyphosate (EPSPS) and HPPD inhibitors was identified with 83% and 27% accuracy, respectively (Figures 6 and 7). Two-way resistance to ALS inhibitors + PS II inhibitors, and glyphosate + ALS inhibitors were identified with 16% and 37% accuracy, respectively (Figure 7). The three-way resistance to glyphosate + ALS inhibitors + PS II inhibitors was identified with 46% accuracy.

Results for HPPD resistance, two-way resistance, and three-way resistance were less consistent, and would require further evaluation to achieve classification accuracies >80%. For future research, classification accuracies of weed species and multiple herbicide-resistant waterhemp biotypes can be increased by integrating NDVI filters on hyperspectral images (Figure 9) and increasing training data collection of plant spectra.

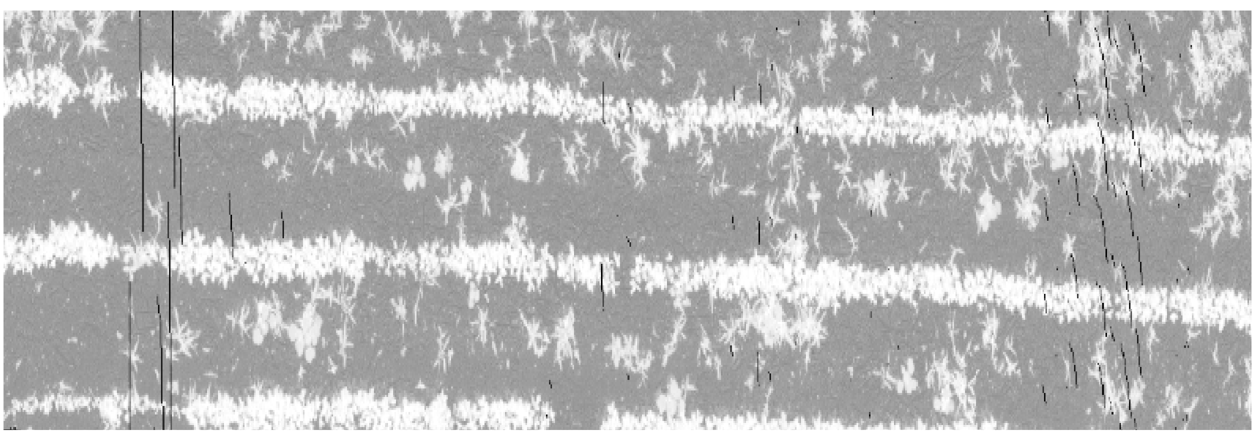

Figure 9. NDVI filter from hyperspectral image in a soybean field

Overall, the results indicate that hyperspectral imaging and neural networks hold promise for early detection of herbicide-resistant weed biotypes in soybean production fields, especially glyphosate-resistant biotypes. This will ultimately lead to development of UAV-based weed maps for timely implementation of integrated weed management (IWM) programs for managing herbicide-resistant weeds in crop production fields.