

Spectral Discrimination of Two Pigweeds from Cotton with Different Leaf Colors

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How to cite this paper: Fletcher, R.S., Reddy, K.N. and Turley, R.B. (2016) Spectral Discrimination of Two Pigweeds from Cotton with Different Leaf Colors. *American Journal of Plant Sciences*, 7, 2138-2150. <http://dx.doi.org/10.4236/ajps.2016.715190>

Received: September 21, 2016

Accepted: October 25, 2016

Published: October 28, 2016

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Abstract

To implement strategies to control Palmer amaranth (*Amaranthus palmeri* S. Wats.) and redroot pigweed (*Amaranthus retroflexus* L.) infestations in cotton (*Gossypium hirsutum* L.) production systems, managers need effective techniques to identify the weeds. Leaf light reflectance measurements have shown promise as a tool to distinguish crops from weeds. Studies have targeted plants with green leaves. This study focused on using leaf hyperspectral reflectance data to develop spectral profiles of Palmer amaranth, redroot pigweed, and cotton and to determine regions of the light spectrum most sensitive for pigweed and cotton discrimination. The study focused on cotton near-isogenic lines created to have bronze, green, or yellow colored leaves. Reflectance measurements within the 400 to 2500 nm spectral range were obtained from cotton and weed plants grown in a greenhouse in 2015 and 2016. Two scenarios were evaluated for the comparison: (1) Palmer amaranth versus cotton lines and (2) redroot pigweed versus cotton lines. Statistical significance ($p \leq 0.05$) was determined with analysis of variance (ANOVA) and Dunnett's test. Sensitivity measurements were tabulated to determine the optimal region of the light spectrum for weed and cotton line discrimination. Optimal bands for weed and cotton separation were 600 to 700 nm (both weeds versus cotton bronze and cotton yellow), 710 nm (Palmer amaranth versus cotton green), and 1460 nm (redroot pigweed versus cotton green). Spectral bands were identified for separating Palmer amaranth and redroot pigweed from cotton lines with bronze, green, and yellow leaves. Ground-based and airborne sensors can be tuned into the regions of spectrum identified, facilitating using remote sensing technology for Palmer amaranth and redroot pigweed identification in cotton production systems.

Keywords

Pigweeds, Cotton Near-Isogenic Lines, Leaf Reflectance

1. Introduction

Cotton growth and productivity in the United States (U.S.) have been negatively impacted by Palmer amaranth and redroot pigweed infestations. Palmer amaranth has reduced cotton yields by 3% to 88% in the U.S. [1] [2] [3] [4] [5]. Cotton yield loss by redroot pigweed infestation ranges from 5% to 90% depending on plant density and soil pattern [6]. Palmer amaranth and redroot pigweed plants produce thousands of seeds that are dispersed by wind, irrigation water, human, and equipment. Seeds germinate throughout the growing season and without proper management, Palmer amaranth and redroot pigweed outgrow cotton plants and become the dominant plants in a cotton field.

Management practices have been established to control and prevent Palmer amaranth and redroot pigweed infestations. The practices focus on pre-emergence and post-emergence strategies. To implement post-emergent strategies effectively, producers need tools to help them identify the locations of Palmer amaranth and redroot pigweed infestations in cotton fields. Ground survey is the standard method, but is tedious and time consuming. Other tools are needed to expedite Palmer amaranth and redroot pigweed identification in cotton production systems.

Multispectral and hyperspectral reflectance data acquired from plant leaves and canopies have shown good potential for differentiating crops from weeds [7]. Using spectral reflectance measurements of plant leaves and canopies, [8] discriminated five weeds [redroot pigweed, common lambsquarters (*Chenopodium album* L.) green foxtail (*Setaria viridis* L.) Beauv., wild mustard (*Sinapis arvensis* L.), and wild oat (*Avena fatua* L.)] and two crops [canola (*Brassica napus* L.) and spring wheat (*Triticum aestivum* L.)], [9] distinguished pitted morning glory (*Ipomea lacunose* L.) from soybean, and [10] differentiated corn caraway (*Ridfolia segetum* Moris.) from sunflower (*Helianthus annuus* L.). Researchers have indicated that approximately 15 to 28 non-redundant spectral bands within visible, red edge, near infrared, and shortwave infrared wavelengths are needed for vegetation study, characterization, and mapping [11].

Weed and crop remote sensing studies have focused on plants with green leaves or compared differences between crops and weeds at different phenological stages. Not all cotton plants have green leaves. For example, cotton near-isogenic lines exist that have bronze, green, or yellow leaves. These plants are currently being evaluated at experiment stations and eventually might be grown in fields. Plant phenology may be ineffective for crop weed discrimination, especially if differences occur at times in which weed management would be the least effective.

Currently, no information is available on spectrally discriminating cotton with different leaf colors from Palmer amaranth and redroot pigweed. The objectives of this study were to develop spectral profiles of Palmer amaranth, redroot pigweed, and cotton with leaf hyperspectral reflectance data, and to determine regions of the optical spectrum most sensitive to pigweed and cotton discrimination. The study specifically focused on differentiating the two pigweeds from cotton near-isogenic lines that have bronze, green, or yellow colored leaves.

2. Materials and Methods

2.1. Experimental Setup

Greenhouse experiments were conducted in 2015 and 2016 at the United States Department of Agriculture-Agricultural Research Service(USDA-ARS) facility (33.425261 latitude, -90.912740 longitude) in Stoneville, MS. Cotton near-isogenic lines created to have bronze, green, or yellow colored leaves, Palmer amaranth, and redroot pigweed were the plants evaluated in this study. Cotton and weed seeds were obtained from seed banks maintained at the laboratory.

A basic description of the cotton near-isogenic lines is as follows. A backcross breeding method was used to develop near isogenic lines for Bronze Leaf cotton from a conventional upland cotton variety. DP 5690 (Monsanto Company, St. Louis, MO; PVPC 009100116) was selected as the wild-type parent; it was developed into a pure inbred line by self-pollination accompanied by single seed descent (SSD) through nine generations using both greenhouse and field plants grown at the USDA-ARS, Stoneville, MS facility. The Bronze leaf parent, "Bronze Leaf" or Seed Accession 31 (SA 30: PI 528567), has a recessive yellow leaf color trait and was obtained from the Mississippi Obsolete Variety Collection. Pollen from SA 31 was used to fertilize emasculated flowers from the SSD DP 5690 inbred genotype. The F_1 seed from each cross was planted in the greenhouse where they were self-fertilized. The F_2 seed were then planted in the field the following spring and the segregating bronze plants were used in five backcrossing events. Five generations of back-crossed seed $BC_{1-5}F_2$ was grown over five consecutive winters and then planted in the field. In the backcross population, pure bronze leaf, yellow leaf, and green leaf were selected and self-pollinated.

Plants were grown in 2-liter plastic pots filled with potting mix (Pro-Mix BX general professional growth medium, Premier Tech Horticulture, Quakertown, PA). Planting dates were May 12, 2015 and May 13, 2016. Plants were subjected to a 14-hr photoperiod; light was provided at the beginning and ending of the day with sodium vapor lamps; the greenhouse temperature was maintained between 21.1°C and 26.7°C. Fertilizer (Dyna-Gro All-Pro 7-7-7, Richmond, CA) and water were added as needed. The experiments consisted of randomized complete block designs with 24 replications to compare the spectral properties of the plants.

2.2. Leaf Reflectance Measurements

Leaf reflectance measurements were obtained with a plant contact probe attached to a spectroradiometer (Fieldspec 3, ASD Inc. Boulder, Colorado) sensitive to a spectral range of 350 to 2500 nm. The contact probe is equipped with a light source, allowing the analyst to collect data at any time during the day or night. Reflectance measurements were obtained from the most recently matured leaf of each plant. The measurements were acquired by attaching the contact probe to the leaf with a leaf clip (ASD Inc. Boulder, Colorado). Data collection was of the upper leaf surface. Instrument reflectance calibration was completed with a white spectralon (white reference) panel prior to the start of data collection and in 15-min intervals. The instrument's software deter-

mined reflectance by dividing the data obtained for a sample by the data obtained for the white reference standard. Reflectance measurements were obtained on June 5, 2015 and June 3, 2016. The goal of weed management strategies is to detect and kill weeds in vegetative growth stages and prior to seeds reaching full maturity levels. Cotton and Palmer amaranth were in the vegetative growth stages and redroot pigweeds were flowering but had not reached full maturity.

2.3. Post-Processing of Reflectance Data

Post-processing of the leaf hyperspectral data was as follows. The 1-nm leaf hyperspectral reflectance data were aggregated to 10 nm spectral bands to reduce the redundancy in adjacent wavelengths [11] [12]. Data aggregation of the leaf hyperspectral reflectance data was completed with the Gaussian distribution function in the *hsdar* (hyperspectral data analysis in R) package [13] of the R software [14]. Then, strong absorption and scatter bands (*i.e.*, 350 - 390 nm, 1360 - 1450 nm, 1800 - 1990 nm, 2360 - 2500 nm) that cannot be used for plant analysis were extracted from the dataset, resulting in 166 spectral bands available for plant analysis.

2.4. Statistical Analyses

Two scenarios were evaluated for statistical analyses: (1) Palmer amaranth versus cotton lines and (2) redroot pigweed versus cotton lines. Statistical analyses included analysis of variance (ANOVA), Dunnett's test, and reflectance sensitivity analysis. ANOVA was used to determine if there was a statistically significant difference ($p \leq 0.05$) among the groups. For the post-hoc test, the goal was to identify the wavelengths in which a statistically significant difference ($p \leq 0.05$) was observed between a specific pigweed group and a specific cotton group. The Dunnett's test was used to achieve that goal.

Reflectance sensitivity analysis [15] was tabulated to determine optimal wavelengths for differentiating Palmer amaranth and redroot pigweed from a cotton group. It was calculated by subtracting mean reflectance of a pigweed group from mean reflectance of a cotton group and then dividing the difference by mean reflectance of the pigweed group. Sensitivity values are positive or negative. Negative values occurred when pigweed mean reflectance values were greater than a cotton group mean reflectance values. Increase or decrease in positive and negative values respectively, indicates an increase in the spectral bands potential for weed cotton separation. Spectral regions in which mean differences were determined to be statistically significant based on Dunnett's test results were also deemed statistically significant for the sensitivity results [15]. Statistical analyses were completed with *base* and *multcomp* [16] packages of the R software.

3. Results

Figure 1(a), **Figure 2(a)**, **Figure 3(a)**, and **Figure 4(a)** show the mean reflectance curves of the pigweeds and the cotton groups. In the 500 to 700 nm region of the spectrum, distinct differences were observed in the amplitude of Palmer amaranth and redroot pigweed reflectance curves versus cotton bronze and cotton yellow reflectance

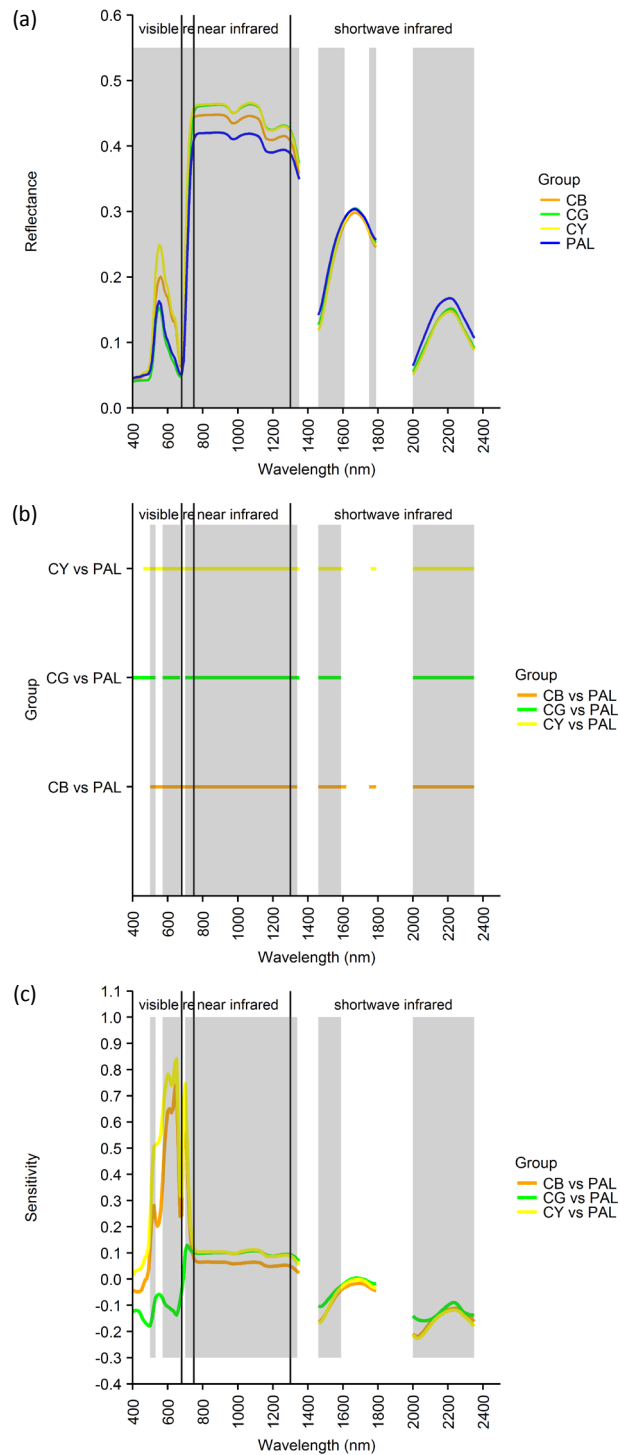


Figure 1. (a) 2015 mean reflectance ($n = 24$) values of cotton bronze (CB), cotton green (CG), cotton yellow (CY), and Palmer amaranth (PAL). Shaded area represents statistical significance ($p \leq 0.05$) based on analysis of variance of treatment data. (b) Colored lines represent Dunnett's test results statistical significance difference ($p \leq 0.05$) for PAL versus (vs) a specific cotton group. Shaded areas—statistically significant difference ($p \leq 0.05$) between PAL and all of the cotton groups based on Dunnett's test results. (c) Sensitivity results of PAL versus (vs) the cotton groups. Shaded areas—same as 1(b). re—red edge.

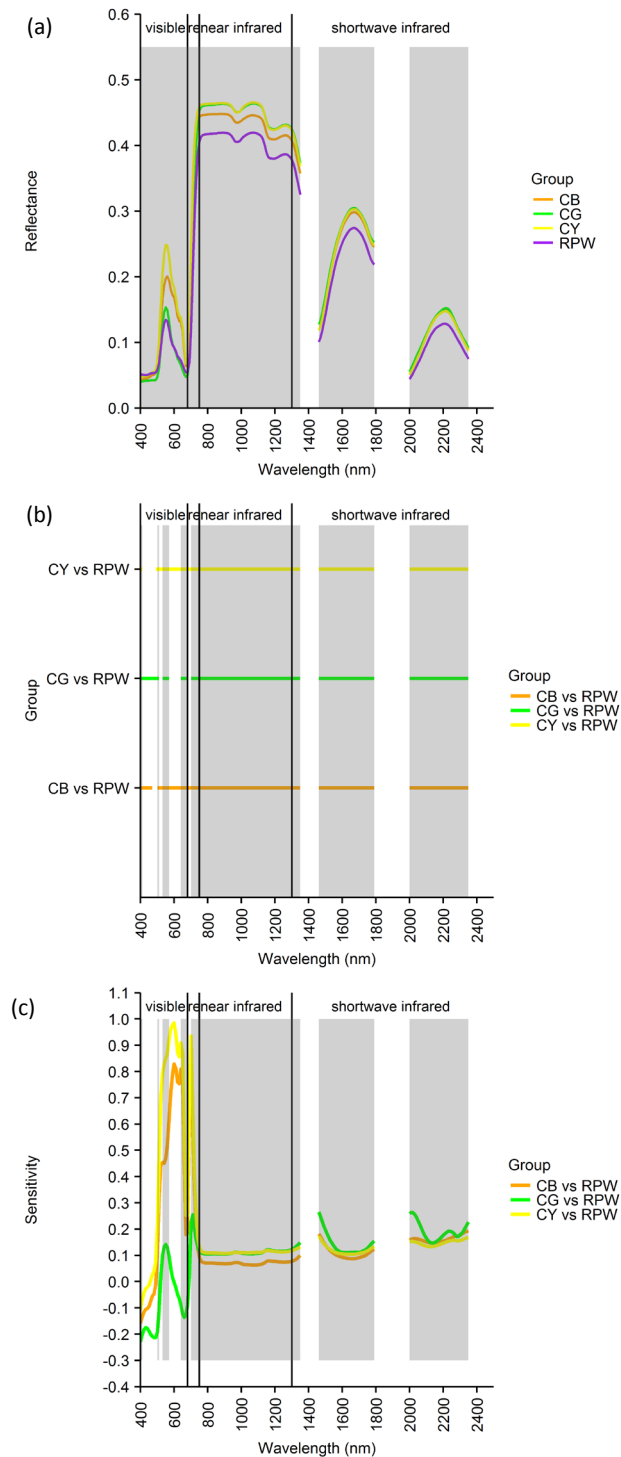


Figure 2. (a) 2015 mean reflectance ($n = 24$) values of cotton bronze (CB), cotton green (CG), cotton yellow (CY), and redroot pigweed (RPW). Shaded area represents statistical significance ($p \leq 0.05$) based on analysis of variance. (b) Colored lines represent Dunnett's test results statistical significance difference ($p \leq 0.05$) for RPW versus (vs) a specific cotton group. Shaded areas—statistically significant difference ($p \leq 0.05$) between RPW and all of the cotton groups based on Dunnett's test results. (c) Sensitivity results of RPW versus (vs) the cotton groups. Shaded areas—same as 2(b). re—red edge.

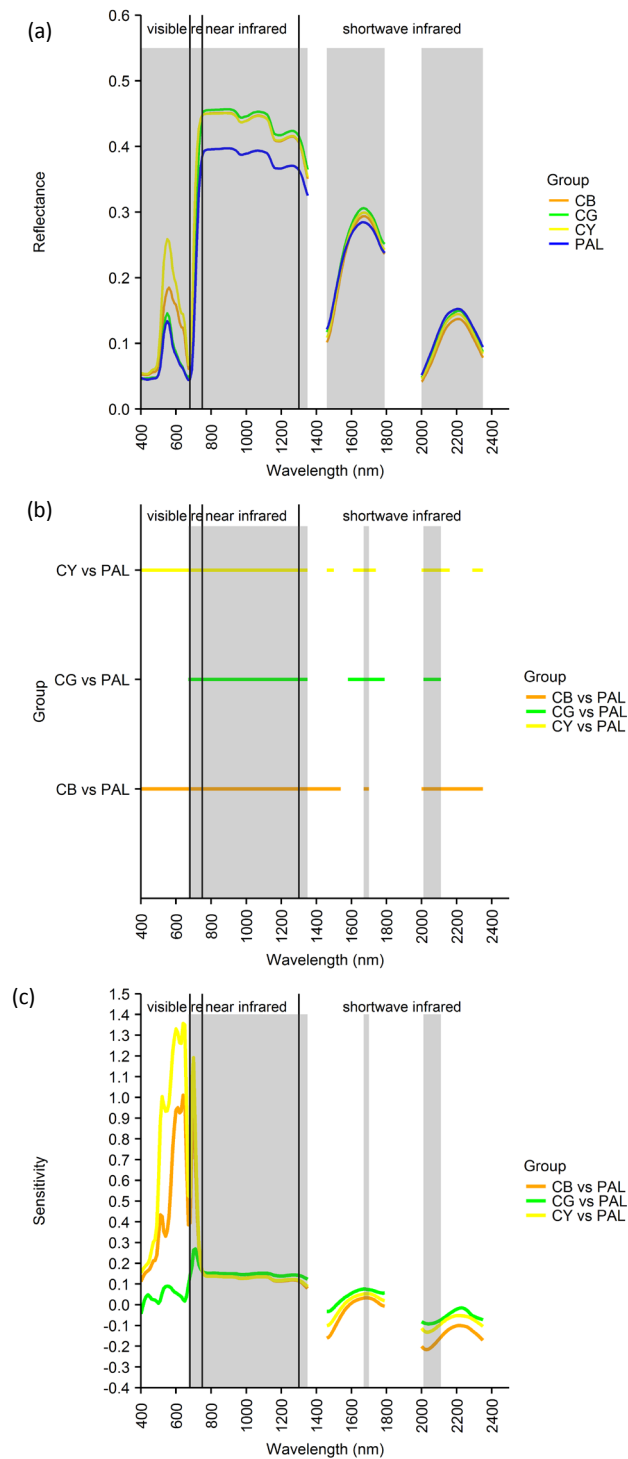


Figure 3. (a) 2016 mean reflectance ($n = 24$) values of cotton bronze (CB), cotton green (CG), cotton yellow (CY), and Palmer amaranth (PAL). Shaded area represents statistical significance ($p \leq 0.05$) based on analysis of variance. (b) Colored lines represent Dunnett's test results statistical significance difference ($p \leq 0.05$) for PAL versus (vs) a specific cotton group. Shaded areas—statistically significant difference ($p \leq 0.05$) between PAL and all of the cotton groups based on Dunnett's test results. (c) Sensitivity results of PAL versus (vs) the cotton groups. Shaded areas—same as (3b). re—red edge.

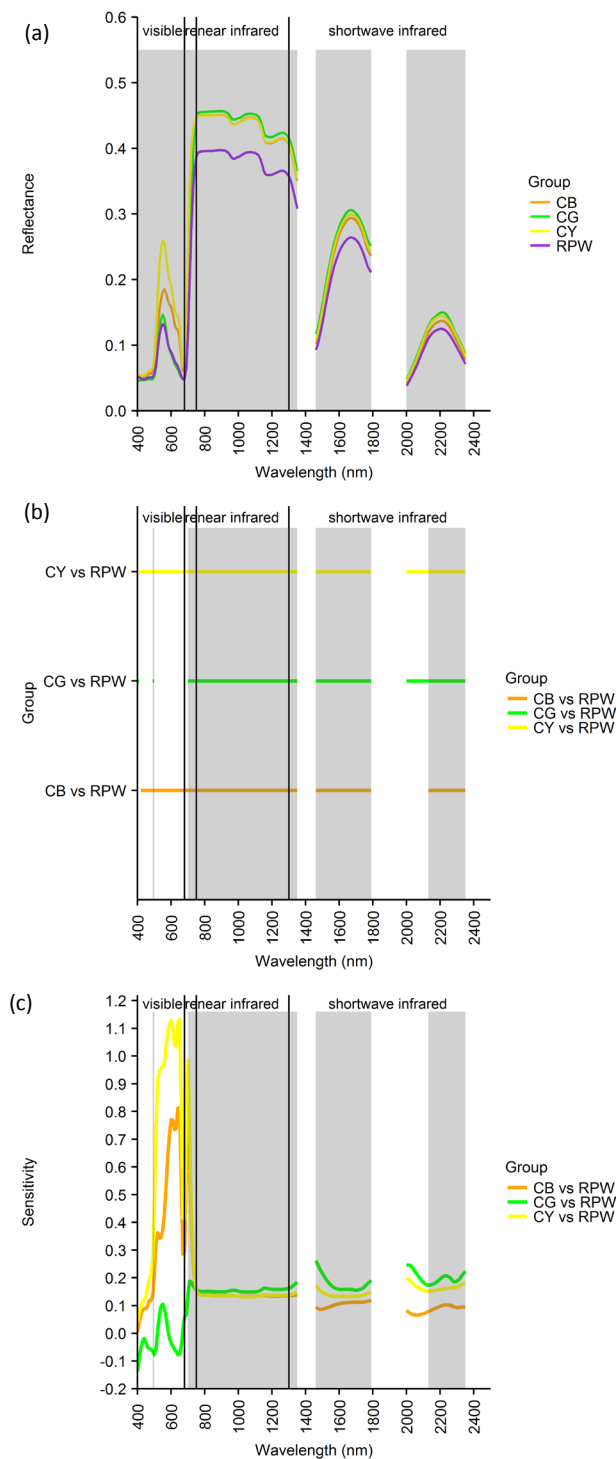


Figure 4. (a) 2016 mean reflectance ($n = 24$) values of cotton bronze (CB), cotton green (CG), cotton yellow (CY), and redroot pigweed (RPW). Shaded area represents statistical significance ($p \leq 0.05$) based on analysis of variance. (b) Colored lines represent Dunnett's test results statistical significance difference ($p \leq 0.05$) for RPW versus (vs) a specific cotton group. Shaded areas—statistically significant difference ($p \leq 0.05$) between RPW and all of the cotton groups based on Dunnett's test results. (c) Sensitivity results of RPW versus (vs) the cotton groups. Shaded areas—same as 4(b). re—red edge.

curves for both years. Also, noticeable reflectance differences occurred between the pigweeds and the cotton groups in the 800 to 1300 nm region of the spectrum. Furthermore, redroot pigweed spectral curves were readily separated from the cotton bronze, cotton green, and cotton yellow spectral curves in the 1600 to 1800 nm region of the spectrum. 92% (153 spectral bands) and 100% (166 spectral bands) of the spectral bands were determined to be statistically significant (ANOVA, $p \leq 0.05$) for the Palmer amaranth-cotton and redroot pigweed-cotton datasets, respectively, in 2015. Statistically significant differences among groups were observed for all of the spectral bands in the 2016 datasets.

Spectral bands within the visible, red edge, near infrared, and shortwave infrared regions of the spectrum were useful for separating Palmer amaranth and redroot pigweed from a cotton group (Figure 1(b), Figure 2(b), Figure 3(b), Figure 4(b) colored lines, Dunnett's test, $p \leq 0.05$). Optimal spectral bands for separating Palmer amaranth and redroot pigweed from cotton yellow and cotton bronze occurred in the 600 to 700 nm spectral range (Table 1, Figure 1(c), Figure 2(c), Figure 3(c), and Figure 4(c)). Peak sensitivity spectral bands of Palmer amaranth and cotton green were identified at 490 nm and 710 nm for the 2015 and 2016 datasets, respectively (Table 1). For both years, peak sensitivity was identified at 1460 nm spectral band for redroot pigweed versus cotton green (Table 1).

Gray shaded areas in Figures 1(b)-4(b) and Figures 1(c)-4(c) represent spectral regions where statistical significance in mean and sensitivity values was observed between a pigweed and all of the cotton groups (e.g., Palmer amaranth versus cotton bronze, Palmer amaranth versus cotton green, and Palmer amaranth versus cotton yellow). If the analyst is willing to accept some loss in sensitivity, the spectral waveband at approximately 710 nm could serve as a universal band for differentiating the pigweeds from the cotton plants.

Table 1. Peak sensitivity of Palmer amaranth and redroot pigweed discrimination from the cotton groups.

Data collection date	Group	Peak sensitivity spectral band
June 5, 2015	Palmer amaranth-cotton bronze	650 nm
	Palmer amaranth-cotton green	490 nm
	Palmer amaranth-cotton yellow	650 nm
	Redroot pigweed-cotton bronze	700 nm
	Redroot pigweed-cotton green	1460 nm
	Redroot pigweed-cotton yellow	600 nm
June 3, 2016	Palmer amaranth-cotton bronze	640 nm
	Palmer amaranth-cotton green	710 nm
	Palmer amaranth-cotton yellow	640 nm
	Redroot pigweed-cotton bronze	650 nm
	Redroot pigweed-cotton green	1460 nm
	Redroot pigweed-cotton yellow	650 nm

4. Discussion

The leaf hyperspectral results support using a multispectral approach to distinguish Palmer amaranth and redroot pigweed from cotton near-isogenic lines with bronze, green, or yellow leaves (**Table 1**, **Figure 1(c)**, **Figure 2(c)**, **Figure 3(c)**, and **Figure 4(c)**). Spectral data within the 600 to 700 nm range was deemed the most sensitive for differentiating Palmer amaranth and redroot pigweed from cotton bronze and cotton yellow. Chlorophyll and carotenoids affect plant leaves reflectance properties in the 600 to 700 nm region of the light spectrum. Chlorophyll strongly absorbs blue (400 to 500 nm) and red light (600 to 670 nm) and moderately reflects green light (500 to 600 nm), resulting in masking of other plant pigments in green leaves. Cotton bronze and cotton yellow plants were designed to have lower chlorophyll levels, thus increasing spectral reflectance of the cotton leaves in the 600 to 700 nm region of the spectrum and allowing other pigments to be seen. Research findings have indicated that it was best to detect yellow to brown foliage in the 680 nm spectral range regardless of the plant stress [15] [17]. The current study did not focus on plant stress per se; nevertheless, the foliage of the cotton plants was yellow green to bronze in color, thus resulting in wavelengths of peak sensitivity being close to the 680 nm spectral range (**Table 1**).

The red edge region, ranging from 680 - 760 nm, is a transitional zone between red and near infrared light reflectance [18] [19]. Therefore, leaf reflectance is a combined response of chlorophyll absorption and internal scattering associated with leaf structure in the red and near infrared regions of the light spectrum, respectively. Increases in chlorophyll cause the red edge region to shift towards shorter wavelengths and vice versa for decreases in chlorophyll content. It was speculated that differences in chlorophyll content caused shifts in the red edge region of the spectrum, leading to the sensitivity peak observed at 700 nm and 710 nm for the 2015 redroot pigweed versus cotton bronze and for the 2016 Palmer amaranth versus cotton green datasets, respectively (**Figure 2(c)**, **Figure 3(c)**). It is also important to note that the 710 nm spectral band maintained its consistency from year to year for the Palmer amaranth versus cotton green dataset, suggesting it was a more reliable band to use compared with the 490 nm spectral band selected for 2015. Finally, the red edge band at 710 nm has strong potential to serve as a universal band for cotton weed discrimination. With this band, the analyst would compromise some sensitivity. However, only a single sensor tuned into that region of the spectrum would be needed for pigweed and cotton separation.

The shortwave infrared region (1300 - 2500 nm) of the spectrum is affected by water content in plants [11] [20] [21] [22]. The sensitivity peak identified in the 1460 nm region of the spectrum for redroot pigweed leaves versus cotton green leaves suggested that redroot pigweed leaves had a higher water content than cotton green leaves (**Figure 2(c)**, **Figure 4(c)**). [23] also indicated that the shortwave infrared region of the spectrum was useful for weed crop discrimination, and [11] indicated the importance of shortwave infrared data for vegetation mapping and crop separation.

All of the cotton groups were readily distinguishable from the pigweeds in the near infrared regions of the spectrum (**Figure 1(a)**, **Figure 2(a)**, **Figure 3(a)**, **Figure 4(a)**);

however, the sensitivity scores of the near infrared spectral bands were intermediate or less than the sensitivity scores of spectral data in other regions of the spectrum (**Figure 1(c)**, **Figure 2(c)**, **Figure 3(c)**, **Figure 4(c)**). Nevertheless, a band selected from that region of the spectrum would provide additional information related to leaf structure [22] to distinguish Palmer amaranth and redroot pigweed from cotton. Any band within the 770 to 1290 nm spectral range would suffice.

Pure leaf spectra and not canopy spectra were used in this study. Therefore, some differences will exist in spectral data collected at the canopy level, which is affected by in-canopy shadowing, leaf orientation, and differences in leaf area. However, the findings provided basic information on leaf reflectance properties of the cotton versus the two pigweeds and on the spectral regions for their differentiation. Additionally, this was the first study in which cotton leaves with different colors were distinguished from Palmer amaranth and redroot pigweed, two troublesome weeds in cotton production systems.

5. Conclusion

Hyperspectral data are effective for developing spectral profiles for pigweeds and cotton, leading to the identification of spectral bands to use on sensors for pigweed cotton discrimination. Optimal spectral bands for pigweed and cotton separation were observed in the 600 to 700 nm spectral range (both weeds versus cotton bronze and cotton yellow), and at 710 nm (Palmer amaranth versus cotton green) and 1460 nm (redroot pigweed versus cotton green). Commercially available cameras can collect data in the wavelengths identified in this study, thus supporting remote sensing as a survey tool for differentiating cotton from Palmer amaranth and redroot pigweed.

Acknowledgements

The authors are grateful to Dr. Vijay Nandula for supplying the pigweed seed and Milton Gaston, Jr., Keysha Hamilton, Arrington Richardson, Arrington Smith, Michael Fisher, Destraile Jackson, Ra Kaiyah Myles, and David Fisher for their assistance in data collection. Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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