

Research Article

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Detection of Palmer amaranth (*Amaranthus palmeri*) and large crabgrass (*Digitaria sanguinalis*) with in situ hyperspectral remote sensing. I. Effects of weed density and soybean presence

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Abstract

The utilization of remote sensing in agriculture has great potential to change the methods of field scouting for weeds. Previous remote sensing research has been focused on the ability to detect and differentiate between species. However, these studies have not addressed weed density variability throughout a field. Furthermore, the impact of changing phenology of crops and weeds within and between growing seasons has not been investigated. To address these research gaps, field studies were conducted in 2016 and 2017 at the Horticultural Crops Research Station near Clinton, NC. Two problematic weed species, Palmer amaranth (*Amaranthus palmeri* S. Watson) and large crabgrass [*Digitaria sanguinalis* (L.) Scop.], were planted at four densities in soybean [*Glycine max* (L.) Merr.]. Additionally, these weed densities were grown in the presence and absence of the crop to determine the influence of crop presence on the detection and discrimination of weed species and density. Hyperspectral data were collected over various phenological time points in each year. Differentiation between plant species and weed density was not consistent across cropping systems, phenology, or season. Weed species were distinguishable across more spectra when no soybean was present. In 2016, weed species were not distinguishable, while in 2017, differentiation occurred at 4 wk after planting (WAP) and 15 WAP when weeds were present with soybean. When soybean was not present, differentiation occurred only at 5 WAP in 2016 and at 3 WAP through 15 WAP in 2017. Differentiation between weed densities did occur in both years with and without soybean present, but weed density could be differentiated across more spectra when soybean was not present. This study demonstrates that weed and crop reflectance is dynamic throughout the season and that spectral reflectance can be affected by weed species and density.

Introduction

With a growing global population to feed, increasing pressure is being placed on agricultural land to be more productive. Despite a steady increase in the global hectareage of arable land since the early 1960s, a sharp decline in the arable land per person has occurred over the same period (FAO 2018). This decline requires increased productivity of arable land. To raise land productivity, negative factors impacting yields must be minimized to meet the population demands for food. A significant contributor to crop yield loss is competition with weeds. Weeds can reduce yield in agronomic crops that contribute to the world food and fiber supply: 79% in soybean [*Glycine max* (L.) Merr.] (Bensch et al. 2003), 91% in corn (*Zea mays* L.) (Massinga et al. 2003), 90% in cotton (*Gossypium hirsutum* L.) (Rowland et al. 1999), and 29% in sorghum [*Sorghum bicolor* (L.) Moench] (Cramer and Burnside 1982).

With the introduction of herbicide-resistant crops and the lack of rotation of herbicide chemistries, the agricultural ecosystem in production fields is changing due to increasing anthropogenic selection pressures on weed populations (Clements and Jones 2021; Owen 2008). This pressure over time has resulted in the selection of weed biotypes that are resistant to herbicides. Globally there are more than 515 species with resistance to at least one herbicidal mode of action (Heap 2021). Some weed species have developed resistance to multiple herbicidal modes of action, making them difficult to control and allowing increased weed competition with

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crops. With resistance on the rise, weeds are often more difficult to control, and growers need to be able to monitor fields, identify weed species and densities, and determine the fluctuation of these weed populations between years. Collecting the aforementioned data on fields will allow growers to implement appropriately timed weed control measures and minimize crop loss due to weed interference. Crop losses associated with weed interference are estimated at \$17 billion for soybean and \$27 billion for corn, roughly 50% of the yield for each of these crops in the United States and Canada (Soltani et al. 2016, 2017). Recent surveys have indicated that Palmer amaranth (*Amaranthus palmeri* S. Watson) was in the top three most troublesome weeds in soybean, with large crabgrass [*Digitaria sanguinalis* (L.) Scop.] being a common weed throughout several other broadleaf cropping systems (Van Wychen 2019). *Amaranthus palmeri* is an erect, multibranched annual weed growing up to 2-m tall, producing greater biomass, and leaf area than other *Amaranthus* species (Bryson and DeFelice 2009; Horak and Loughlin 2000). *Digitaria sanguinalis* is a prostrate to spreading summer annual with a height of 0.7 m (Bryson and DeFelice 2009; Holm et al. 1991). Both species have high levels of fecundity and competition in cropping systems (Basinger et al. 2019; Norsworthy et al. 2016; Peters and Dunn 1971). Concerns from the public about weed resistance, environmental impacts of pesticides, and possible restrictions on herbicide use may force new management strategies for weeds.

To minimize direct or indirect crop loss to weeds, weed management is critical. One approach to weed management is the use of site-specific weed management. Site-specific weed management is designed to control weeds only where they are present and to reduce the environmental impacts of herbicide applications and tillage. Site-specific management can reduce herbicide inputs, soil compaction, energy consumption, and off-target herbicide application (Brown and Noble 2005; Coleman et al. 2019; Fernandez-Quintanilla et al. 2018). Site-specific management requires accurate scouting of fields to determine whether weed management is needed or to accurately detect and identify weeds (Hunter et al. 2019; Li et al. 2021). In agronomic crops, weed management must take place early in the season to minimize the interference weeds have in the crops. To do so, scouting for weeds must be done at targeted timings to determine the appropriate time for the implementation of weed control. Recommendations resulting from a scouting exercise are frequently tied to the quality of the scouting data, and accurate scouting can be time-consuming. Remote sensing may provide a solution for accurate and timely scouting of agricultural fields. Remote sensing has been utilized in agriculture to predict yield and biomass (Chen et al. 2018; Yue et al. 2021), determine crop nutrient or water stress (Basso et al. 2016; Bellvert et al. 2014; Mahajan et al. 2017) and crop-weed competition (Ronay et al. 2021), and detect the presence of insects or plant disease (Abudulridha et al. 2020; Franke and Menz 2007; Rhodes et al. 2022). Remote sensing for weed detection has been attempted since the early 1980s (Menges et al. 1985; Richardson et al. 1985). These attempts at weed detection, along with later attempts (Brown et al. 1994; Everitt et al. 1987, 1992; Medlin et al. 2000; Yang and Everitt 2010), were successful in discriminating weeds but unable to connect discrimination to management decisions.

Research focused on remote sensing of weeds and invasive species has used satellite data or data collected using aerial sensors (Hunt et al. 2007; Menges et al. 1985). These methods often lack the spatial and temporal resolution to detect weeds intermixed with crops. Some of these limitations have been overcome through

the use of unmanned aerial vehicles (UAVs); however, there are still limitations to this technology (Huang et al. 2018). Additionally, many studies have been conducted using spectrally limited sensors and may only contain wavelengths in the visible (VIS) or shortwave portions of the near-infrared (NIR) spectrum. The use of hyperspectral remote sensing has allowed for discrimination of weed species (Gray et al. 2009) and detection of herbicide drift (Huang et al. 2016; Suarez et al. 2017) in agricultural settings. Hyperspectral data provide greater spectral resolution and could allow for the detection of differences between crop and weed species while also detecting biophysical differences. Research utilizing hyperspectral data that account for weed density and crop/weed phenology as a means of weed detection is limited. Studies examining reflectance spectra often are conducted in only a single year (Koger et al. 2004b) or have limited temporal data-collection dates (Goel et al. 2003; Hunt et al. 2007).

Recent approaches have begun to bridge the gaps between data collection, weed discrimination, and management decisions through the aggregation of technologies. The implementation of UAVs and other technologies for the discrimination of weeds must rely on in situ data collection to ground-truth UAV data (Huang et al. 2016; Shafian et al. 2018; Suarez et al. 2017) and elucidate relationships not detected by sensors on a UAV. The integration of the information from these technologies not only improves the discrimination of weeds from crops but allows for the implementation of real-time control measures (Hu et al. 2020; Hunter et al. 2019). Despite the best efforts to integrate these technologies, research using in situ remote sensing is needed to continue to improve the accuracy of current and future hyperspectral UAV and ground-based weed management systems. These systems could be greatly improved by identifying novel reflectance regions that could be leveraged for species discrimination and detection. Thus, the objectives of this study are to determine (1) whether weed species can be differentiated in situ, (2) the effect of soybean and weed phenology on differentiation, (3) the effect of weed species and density on hyperspectral reflectance, and (4) the effect of crop presence on weed detection and density differentiation.

Materials and Methods

Field studies were conducted on 'AG6536' soybean in 2016 and 2017 at the Horticultural Crops Research Station near Clinton, NC (35.0242°N, 78.2828°W). The studies were conducted on a Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiodults) with 0.31% humic matter and pH 5.9 and an Orangeburg loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiodults) with 0.47% humic matter and pH 5.9 in 2016 and 2017, respectively.

Experimental Design and Treatments

'AG6536' soybean was planted at a seeding rate of 321,000 seeds ha⁻² (10-cm in-row spacing) using a four-row vacuum planter on June 9, 2016, and June 12, 2017. Treatments were combinations of crop presence or absence, weed species (*A. palmeri* or *D. sanguinalis*), and weed density (1, 2, 4, 8 and 1, 2, 4, and 16 plants m⁻², respectively) arranged in a randomized complete block design with three replications. Plots consisted of 4 rows, each 30-cm wide by 5-m long. The day following crop planting, designated plots were seeded by hand with each weed species. In plots designated as no crop, soybean was pulled upon emergence. *Amaranthus palmeri* at approximately 8-cm tall and *D. sanguinalis* at two expanded

leaves were thinned to 1, 2, 4, 8 and 1, 2, 4, and 16 plants m^{-2} , respectively, then maintained weekly using hand removal. Additionally, a plot containing no weeds served as a weed-free check plot, and a plot containing no crops and no weeds was utilized as a bare-ground treatment.

Data Collection

Spectral data were collected across five dates in 2016 and six dates in 2017 to determine the effect of phenology on spectral variability and for detection of weed species and density. Spectral measurements were collected using a spectrometer (PSM-2500, Spectral Evolution, 1 Canal Street, Lawrence, MA 01840) with fiber optic capable of a 44-rad field of view ($0.44 m^{-2}$ ground field of view for each reading), between 1000 and 1400 hours, in full sunlight. Spectra were collected and graphed at the time of collection using data-acquisition software (Darwin SP, Spectral Evolution). The spectrometer has a spectral resolution of 3.5 nm at 700 nm, 22 nm at 1,500 nm, and 22 nm at 2,100 nm. Data output is in 1-nm increments, resulting in 2,151 bands reported. To account for environmental and atmospheric variability during each data-collection date, the sensor was calibrated every 15 min using a white reflectance panel (Spectralon, Labsphere, 231 Shaker St, North Sutton, NH 03260). Spectrometer measurements were taken at nadir. A meter stick was placed at the apex of the average plant height in the plot to ensure that all measurements were taken 1 m over the plot canopy. Five measurements were taken for each plot to capture crop and weed variability across the plot area, including soil background, with a plot with no soybean and no weeds serving as a bare-ground plot for soil reference. The bare-ground plot served as a negative control to ensure that spectra from crops, weeds, mixed spectra of crops, and weeds were able to be differentiated from the soil. Crop and weed phenology and heights were determined based on methods by Meier (2018) at each spectral data collection.

Data Processing

Hyperspectral data for each plot and date were graphed using the GGPlot2 package in R software (v. 3.4.2) (Wickham 2013). Visual quality control was performed for each graph, and data containing interference or noise as noted in field notes were removed. Reflectance spectra were then grouped by date, weed type, and the presence or absence of crop. Each subset of data was subjected to the Kruskal-Wallis test to determine overall groupwise differences at each of the 2,151 bands reported (Corder and Foreman 2009). Comparisons were made by wavelength band to determine species differentiation. To determine differences within each subset of data, differences at each reported band were tested using the Mann-Whitney *U*-test (Corder and Foreman 2009; Schmidt and Skidmore 2003). The null hypothesis being tested states that median reflectance for each reported band is not different between weed species or density. Levels of significance were set at $P \leq 0.1$ due to limits in sample size and to explore a range in which additional wavelengths may provide confidence in the differentiation of weed species and density. *P*-values are reported as a continuous variable for each wavelength, where $P \leq 0.1$. In an agricultural setting like the one in which this study was conducted, outcomes of misidentification would result in mistaking a crop plant for weed or vice versa. If site-specific weed management is the goal, discrimination of weeds at a $P \leq 0.1$ level would prove beneficial for use in a weed control program.

Results and Discussion

Differentiation of All Treatments

Throughout the rest of this paper, results for spectra will be discussed corresponding to the following spectral regions: visible (VIS = 350 to 700 nm), near-infrared (NIR = 700 to 1,300 nm), shortwave-infrared region 1 (SWIR1 = 1,500 to 1,900 nm), and shortwave-infrared region 2 (SWIR2 = 1,900 to 2,500 nm). The shortwave-infrared spectral region was subdivided into SWIR1 and SWIR2 to provide more meaningful results given the large spectral range of the SWIR region.

Groupwise differentiation including all combinations of weed species, weed density, and crop presence or absence were compared with one another (Figures 1 and 2). Spectral reflectance was different across years and the varying crop and weed phenological time points at which the spectra were collected. Therefore, data are presented by year and weeks after planting (WAP). In 2016, differentiation between weed species, density, and crop presence was most prevalent across spectra at 5 WAP. The differentiation at this timing was when there was the greatest difference in vegetation cover between the plots containing soybean and the plots containing only weeds. Plots containing lower weed densities at this timing were more spectrally similar to the bare-ground plots. At this point in the season, weeds canopies were not large enough to fill the entirety of the plots as they did late season. For plots containing soybean or higher weed densities, spectra were more similar due to greater amounts of vegetation when compared with the bare-ground and lower weed density plots. This trend was similar in 2017 at 3, 4, and 5 WAP (Figure 2).

Other spectral differences in 2016 were seen at 4, 7, and 11 WAP and were confined to spectral reflectance and absorption magnitude differences in the VIS (Figure 1). This was due to the inclusion of the bare-ground treatment, for which differences were observed in spectral magnitude in the VIS and SWIR1 and SWIR2. In 2017, however, differences were seen across the VIS and both SWIR1 and SWIR2 from 3 WAP to 15 WAP. At 15 WAP, additional spectra of interest were noted in the NIR region (Figure 2). Early-season differentiation was not possible between weeds and bare ground at 2 and 3 WAP in 2016 and 3 WAP in 2017. At this time point, weeds are at an optimal stage for control using chemical or mechanical control measures due to their small size (<10 cm). During both years, there were differences in spectral magnitude that are associated with changes in the type (weed species vs. crop) and amount (weed density) of vegetation present. Furthermore, spectral reflectance curves were not constant between reading dates, as these curves change in magnitude and shape with changing plant phenology, crop presence, and weed species and density

Differentiation of Weed Species

In 2016, weed species could not be differentiated from one another regardless of species or weed density in the presence of soybean (Figure 3). Soybean reduced weed biomass (Basinger et al. 2019), which could have affected the ability to have spectral differences between both weed species and weed density. Similar results were seen without soybean present; only a few spectra in the blue range at 5 WAP showed differences between weed species (Figure 4). When compared with bare ground, all of the plots containing weeds at any density had greater absorbance in the blue region. The blue region of the spectrum is associated with greater amounts of chlorophyll *a* and chlorophyll *b* as well as β -carotene (Jensen 2006; Mahlein et al.

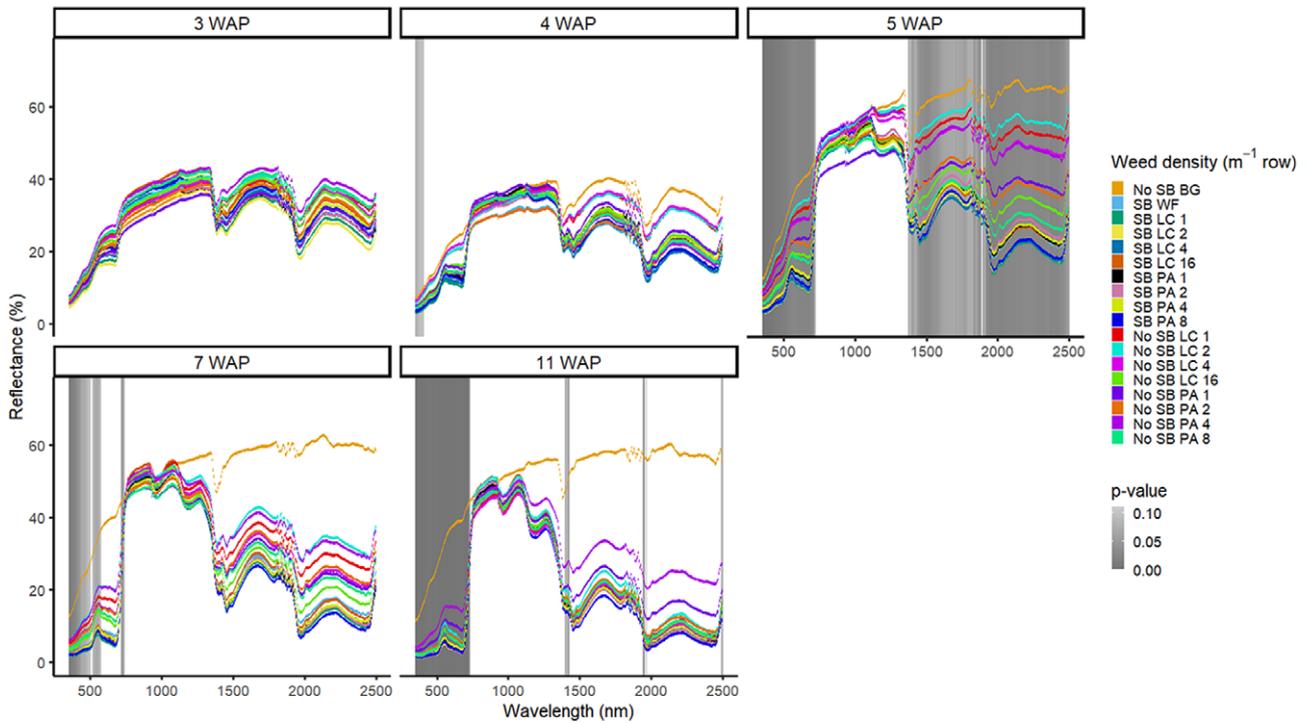


Figure 1. Spectral reflectance for all *Amaranthus palmeri* (PA) and *Digitaria sanguinalis* (LC) densities with (SB) and without soybean at the Horticultural Crops Research Station, Clinton, NC, 2016. Bare ground (No SB BG) and weed-free soybean (SB WF) were grouped by weeks after planting (WAP).

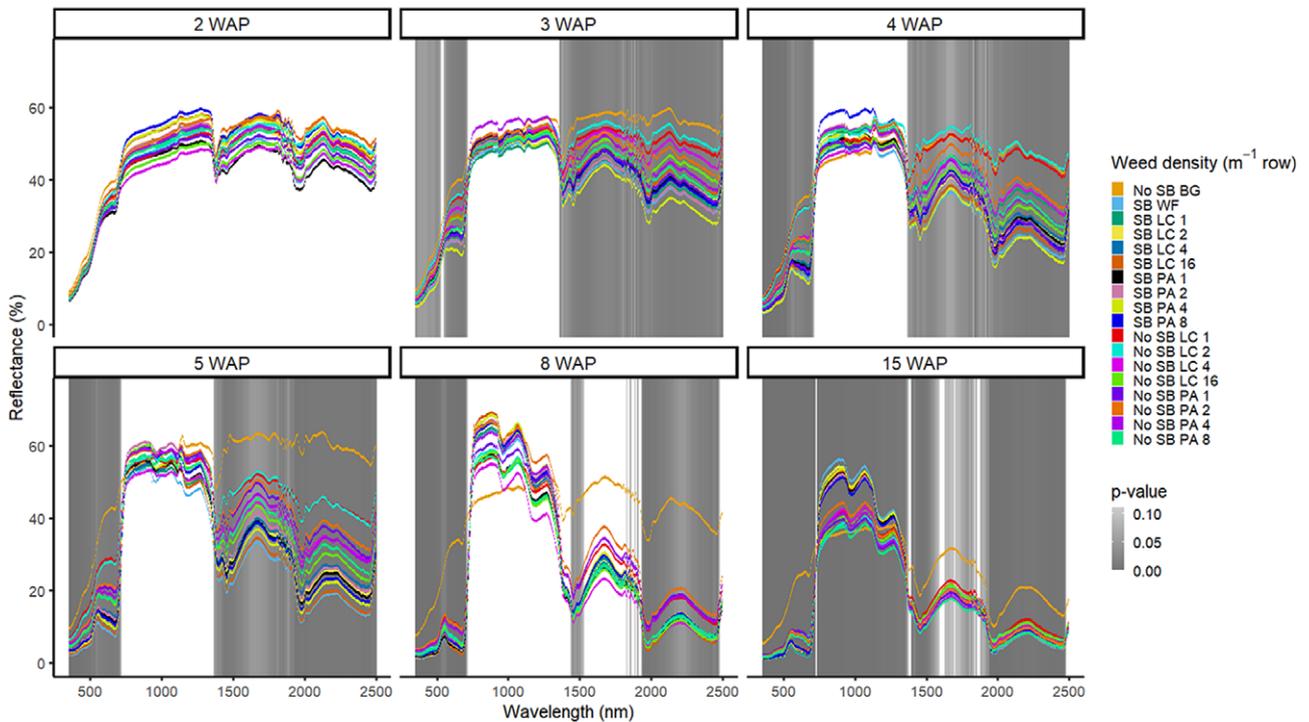


Figure 2. Spectral reflectance for all *Amaranthus palmeri* (PA) and *Digitaria sanguinalis* (LC) densities with (SB) and without soybean at the Horticultural Crops Research Station, Clinton, NC, 2017. Bare ground (No SB BG) and weed-free soybean (SB WF) were grouped by weeks after planting (WAP).

2015), which would not be present in a plot with no vegetation. In 2017, results showed differences between weed species with soybean in blue spectra at 4 WAP and in the VIS and SWIR2 at 15 WAP (Figure 5). Differences in SWIR2 could be related to leaf water content or caused by competition within and between

species, as greater reflectance could be a result of lower relative water content (Carter 1991). Without soybean present, differences between species were detected across more spectra in the VIS at 3 to 15 WAP and in the SWIR1 and SWIR2 at 8 to 15 WAP (Figure 6). These late-season differences in the

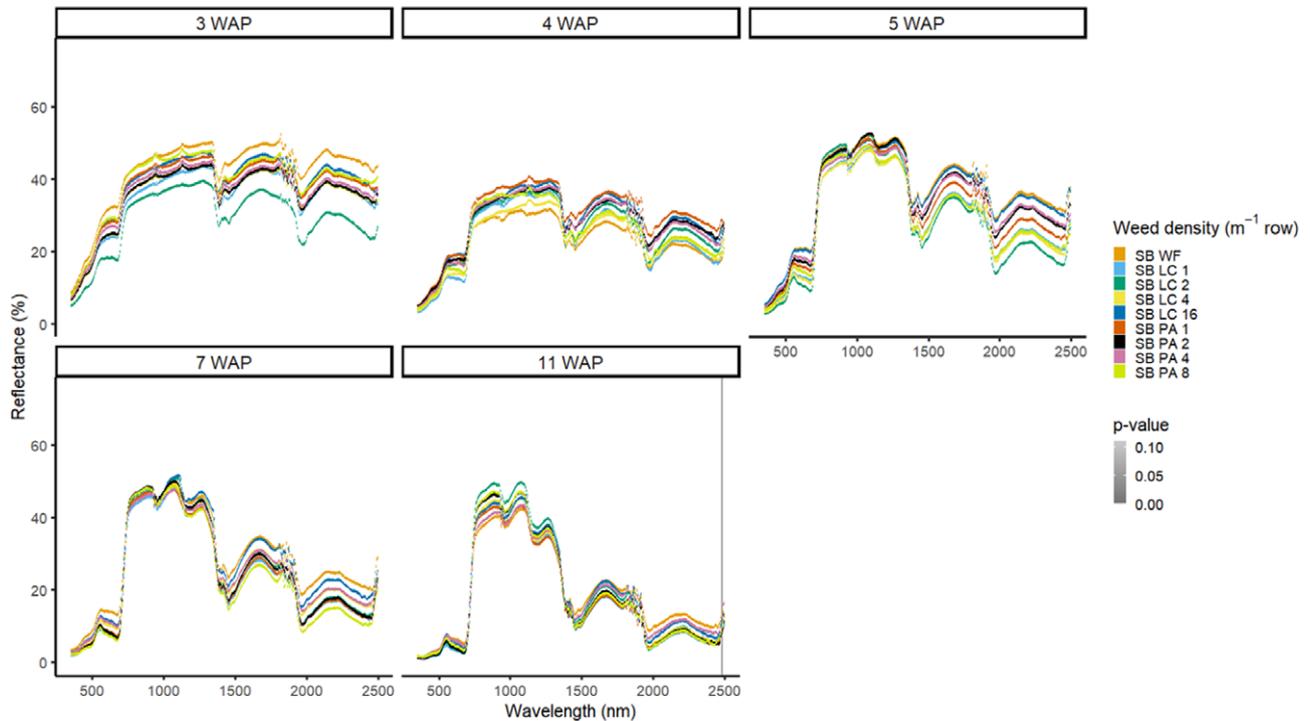


Figure 3. Spectral reflectance for all *Amaranthus palmeri* (PA) and *Digitaria sanguinalis* (LC) densities with soybean (SB) and a weed-free control (SB WF), grouped by weeks after planting (WAP), at the Horticultural Crops Research Station, Clinton, NC, 2016.

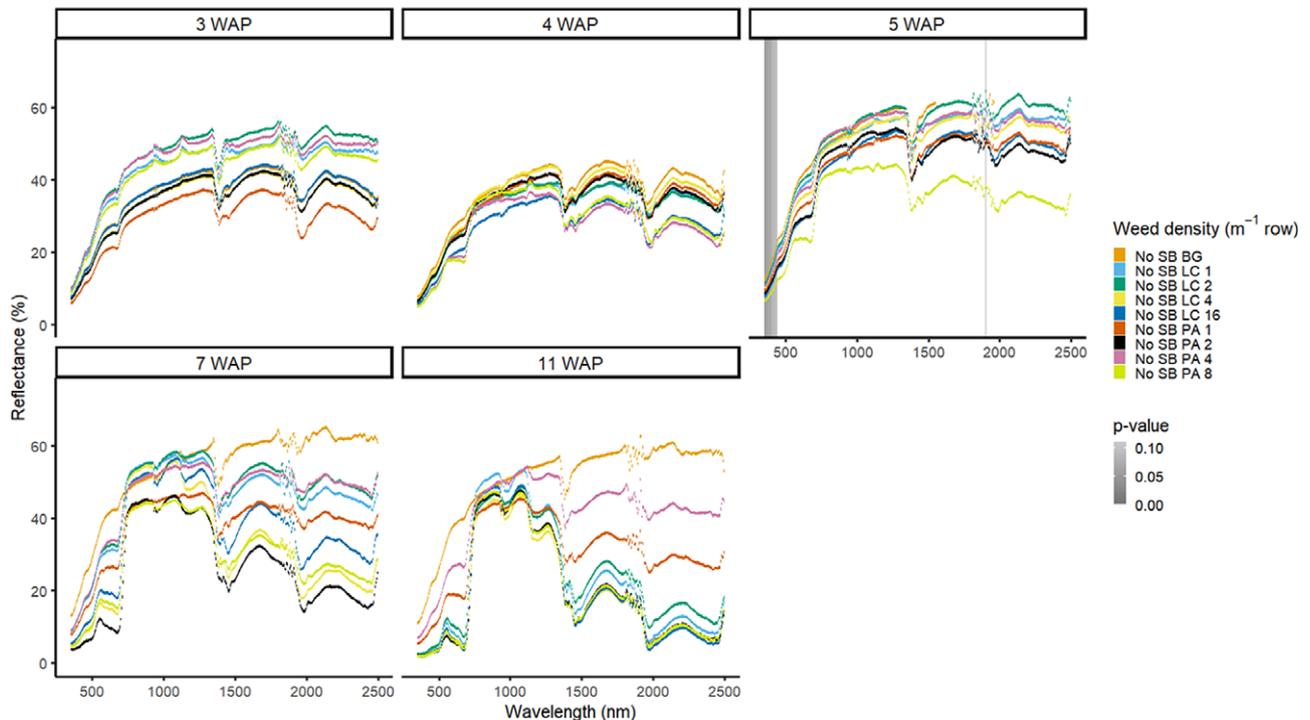


Figure 4. Spectral reflectance for all *Amaranthus palmeri* (PA) and *Digitaria sanguinalis* (LC) densities without soybean as a bare-ground control (No SB) grouped by weeks after planting (WAP), at the Horticultural Crops Research Station, Clinton, NC, 2016.

SWIR regions are tied to differences between the bare-ground plots and weed species and density due to the increased presence of foliage.

Results from this study show that spectral differentiation can occur between weed species and weed density. Successful

discrimination of species in both agricultural and nonagricultural environments has previously been reported (Henry et al. 2004; Koger et al. 2004a; Santos et al. 2011; Schmidt and Skidmore 2003; Ustin et al. 2009). However, the inconsistency of spectral differentiation across years and phenological time points complicates

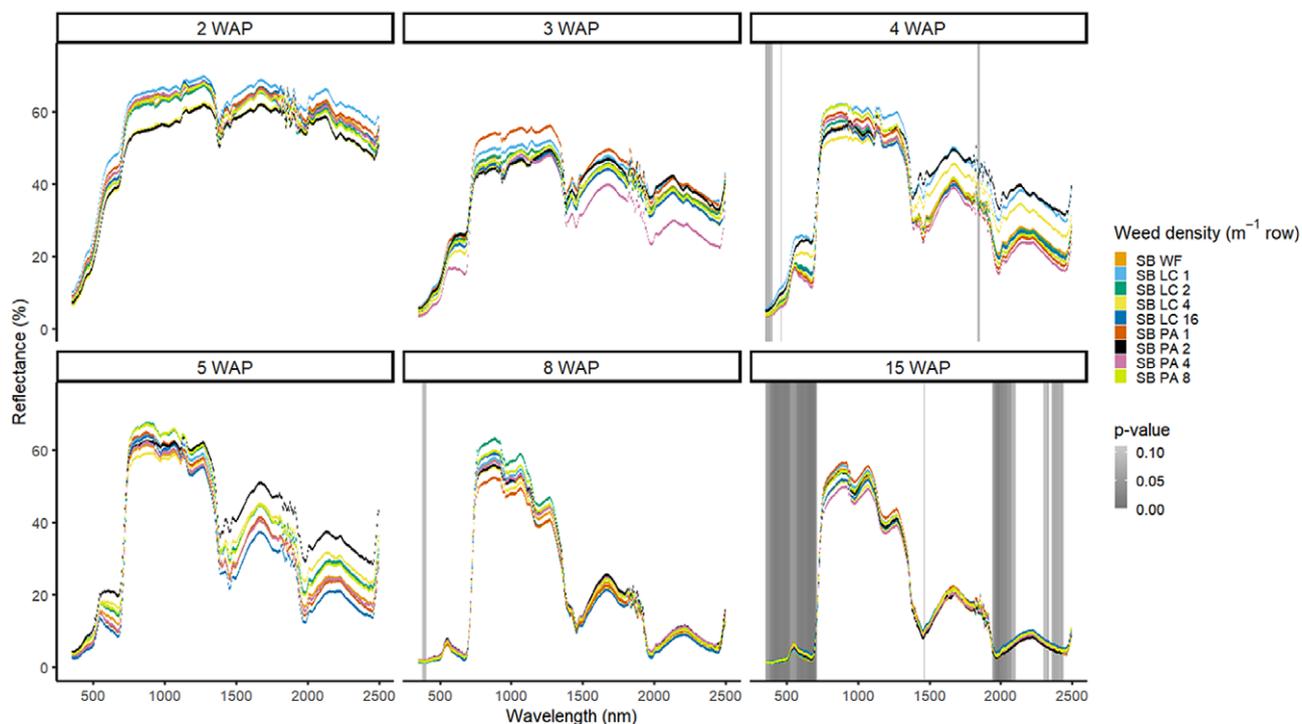


Figure 5. Spectral reflectance for all *Amaranthus palmeri* (PA) and *Digitaria sanguinalis* (LC) densities with soybean (SB) and a weed-free control (SB WF), grouped by weeks after planting (WAP), at the Horticultural Crops Research Station, Clinton, NC, 2017.

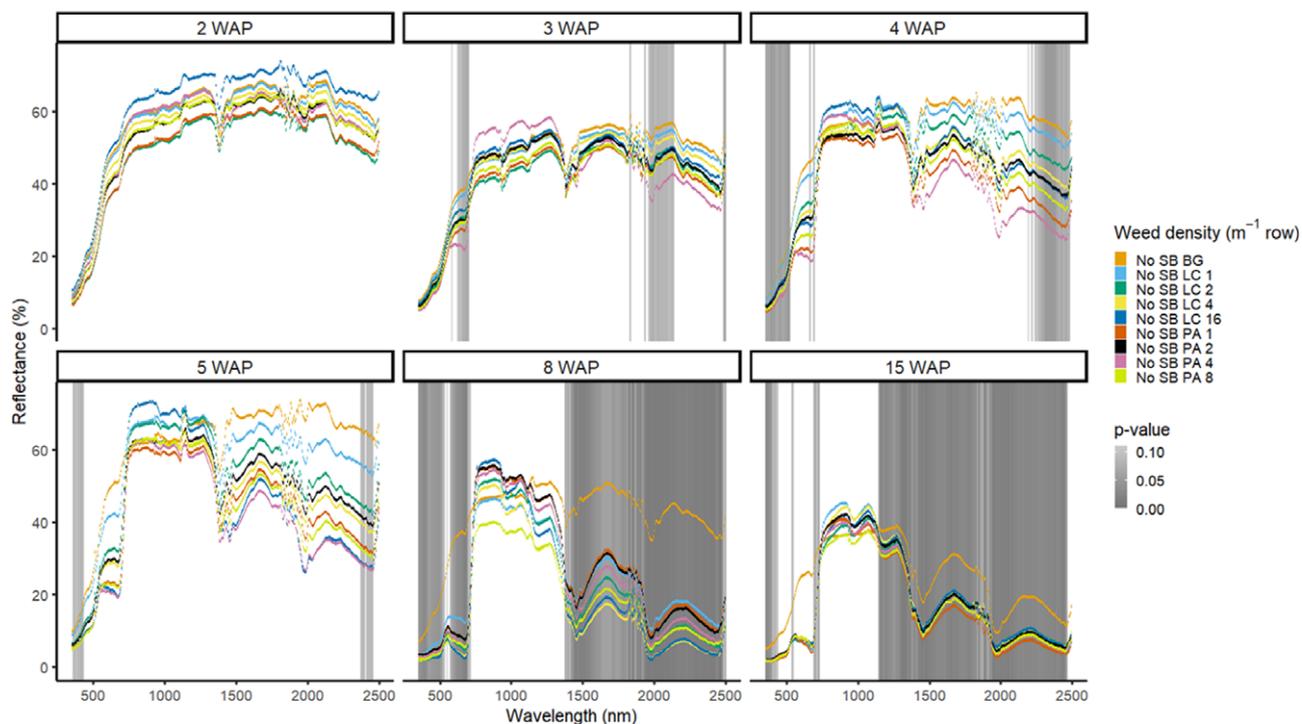


Figure 6. Spectral reflectance for all *Amaranthus palmeri* (PA) and *Digitaria sanguinalis* (LC) densities without soybean as a bare-ground control (No SB BG) grouped by weeks after planting (WAP), at the Horticultural Crops Research Station, Clinton, NC, 2017.

discrimination between species. Differences in environmental conditions from year to year may alter phenology, and spectra may not be uniform from year to year (Table 1). These differences may have caused spectral variation due to variable rainfall or other environmental changes between seasons.

Weed species differentiation had limited spectra for weed species separation when in the presence of a crop and was not successful at low weed densities. The lack of differentiation at lower densities is likely due to the mixture of crop and limited weed species canopy. Weed biomass was lower when crops were present,

Table 1. Mean monthly temperature, growing degree days (GDD), and precipitation for Clinton Horticultural Crops Research Station, Clinton, NC, from June to November for 2016 and 2017.

Month	Average monthly temperature		GDD		Precipitation	
	Year		Year		Year	
	2016	2017	2016	2017	2016	2017
	C		base 10 C		mm	
June	25	24	473	437	93	150
July	28	27	568	527	154	86
August	27	26	538	493	107	125
September	24	23	437	393	287	132
October	18	18	274	272	281	53
November	12	11	93	74	20	31

and weed species at low densities often had limited canopy coverage over the crop, limiting their spectral contribution to the mixed spectra. (Basinger et al. 2019). Greater weed densities had higher biomass per square meter and contributed more to the mixed spectra. At the highest weed density, each species was more readily differentiated compared with the lower densities, due to increased weed canopy reflectance. Differentiation of weed species occurred later than would be acceptable for implementing chemical control of *A. palmeri*, but control options for *D. sanguinalis* would still be available.

Weed Density

Weeds with Soybean

The four densities of *D. sanguinalis* in the presence of soybean were not spectrally different from one another in 2016, except for only a few spectra in the NIR at 4 WAP (Figure 7). It would not be too late to control *D. sanguinalis* in soybean at 4 WAP (Van Acker et al. 1993). However, this difference at 4 WAP was not consistent between years and could not be relied on for making a management decision. In 2017, differences of *D. sanguinalis* with soybean were different at 4 WAP in the blue region of the VIS, and at 15 WAP throughout the VIS and in portions of the SWIR2 (Figure 8). The differences in blue regions at this time were likely tied to chlorophyll content at this time of the season (Jensen 2006; Mahlein et al 2015) However, the differences seen at 15 WAP were due to *D. sanguinalis* completing its life cycle and beginning to senesce and plant desiccation occurring while soybean was not physiologically mature. If *D. sanguinalis* was not detected, interference at the densities in this study could result in yield losses of up to 38% at 16 plants m⁻² (Basinger et al. 2019).

Weed density with soybean present was more easily detected for *A. palmeri* than for *D. sanguinalis*. In 2016, in the presence of soybean, densities of *A. palmeri* were only different at limited spectra in the NIR at 4 WAP (Figure 9). All plots containing *A. palmeri* had greater reflectance in the NIR, which likely resulted from greater overall vegetation coverage at this point in the season (Table 2). In 2017, with soybean, spectral differences only occurred at 4 and 15 WAP in the VIS (Figure 10); differences at 15 WAP were likely due to the presence of reproductive structures in the plots containing *A. palmeri* and the beginning of senescence of the weeds in the plots. At 15 WAP, pairwise comparisons between the weed-free treatment and all weedy treatments regardless of density showed differences within the VIS (data not shown). In a previous study, the yield loss due to the presence of *A. palmeri* at these

densities resulted in up to 37% yield loss at 8 plants m⁻² (Basinger et al. 2019). Unfortunately, by 15 WAP, *A. palmeri* had begun to senesce, contributing seeds to the soil seedbank, and no control measure would be efficacious during this time during the season (Spaunhorst et al. 2018).

Weed biomass reduction in soybean is likely the reason for poor discrimination of weed species and density (Basinger et al. 2019). This study showed that weed biomass tended to be significantly greater when not grown with soybean and tended to increase with increasing density. This is likely to be a significant contributing factor to the limited spectral differences seen in the present study. Furthermore, increased leaf layers due to the multi-scaffold branched trifoliolate leaves produce many leaf layers, increasing radiative transfer for soybean. This type of structure is similar to that of *A. palmeri*, making the differentiation of the two species difficult. *Amaranthus palmeri* was detected in this system when the canopy became established more quickly than soybean and was detectable in the VIS and NIR. *Amaranthus palmeri* was more readily detected at low densities due to a quickly establishing tall broadleaf canopy that was present above the soybean canopy. However, once the soybean canopy became established, determination of *A. palmeri* density became more difficult due to the increased leaf area of the crop.

Digitaria sanguinalis was difficult to detect in soybean, and soybean did not allow for large numbers of *D. sanguinalis* leaves to emerge through the soybean canopy. Detection of *D. sanguinalis* occurred during the onset of this weed's reproductive structures, which were able to protrude through the soybean canopy (Figure 11).

Estimation of plant density using hyperspectral remote sensing has been successful (Shafian et al. 2018; Thorp et al. 2008). Detection of *A. palmeri* occurred early in the season due to rapid biomass accumulation, as seen in previous research (Horak and Loughlin 2000). *Digitaria sanguinalis* was slower establishing a canopy, which made it more difficult to detect early season. Density differentiation between weed-free and lower weed densities has been demonstrated in soybean by others (Koger et al. 2004b). However, these differences were not detectable in the presence of soybean in large regions of spectra.

Weeds without Soybean

Detection of weed density without a crop present was more consistent in identifying large portions of spectra that allowed for density discrimination. Detection of both weeds, when compared with the bare-ground control, occurred earlier for both species when density was highest. In 2016, when soybean was not present, *D. sanguinalis* had more spectra distinguishable based on density at 5 to 11 WAP (Figure 12). Interestingly, it was not until 5 WAP that *D. sanguinalis* could be distinguished from bare ground, and then only in the VIS regions. Furthermore, for spectral reflectance in the VIS, SWIR1, and SWIR2, the only regions where differences were detectable, reflectance magnitude was related to *D. sanguinalis* density, with greater densities (4 and 16 plants m⁻²) having greater spectral absorbance from 7 to 11 WAP. This is somewhat problematic, as the *D. sanguinalis* plants were quite large at 20-cm tall, having at least 3 internodes visible (Table 2), making it challenging to control at this size. In 2017, when soybean was not present, differences in *D. sanguinalis* density were noted at 3 WAP on the red edge and the SWIR2 (Figure 13). However, more spectra were differentiable at 4 and 5 WAP and in the blue and longer SWIR spectra. At 3 to 8 WAP, the trend of greater absorbance with greater density was similar to that seen in 2016,

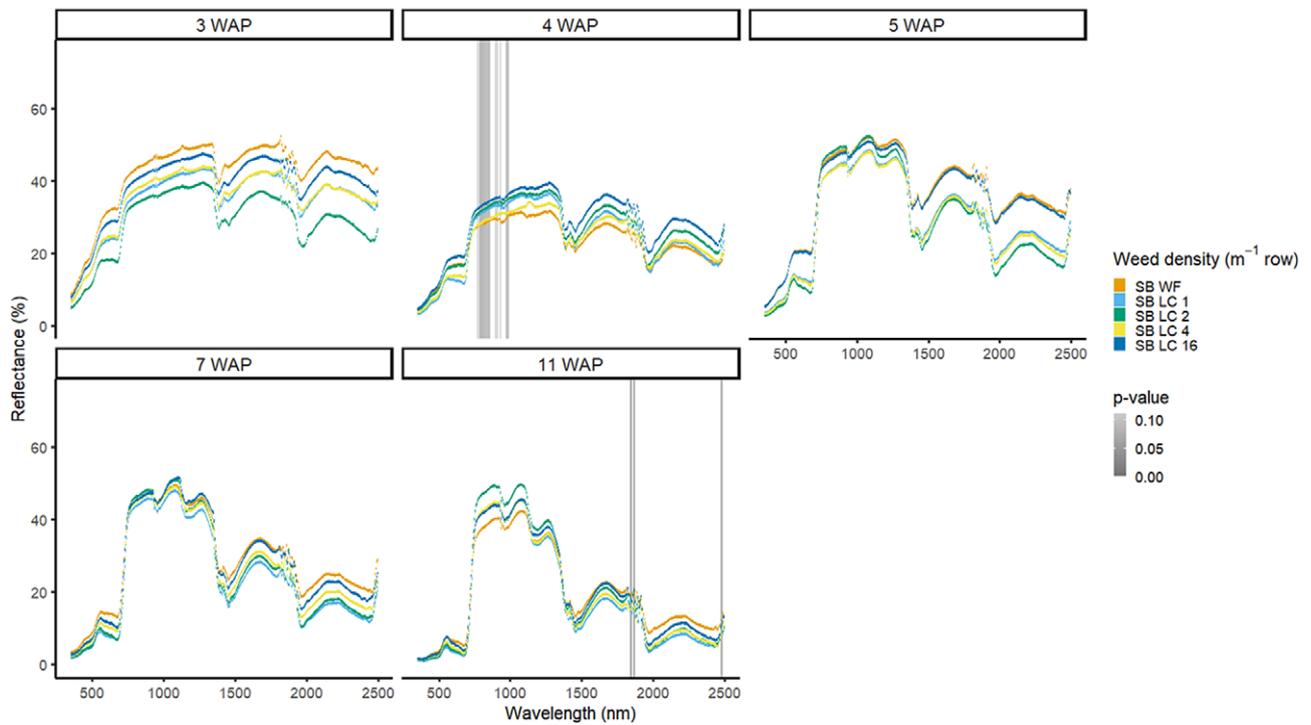


Figure 7. Spectral reflectance for all *Digitaria sanguinalis* (LC) densities with soybean (SB) and a weed-free control (SB WF), grouped by weeks after planting (WAP), at the Horticultural Crops Research Station, Clinton, NC, 2016.

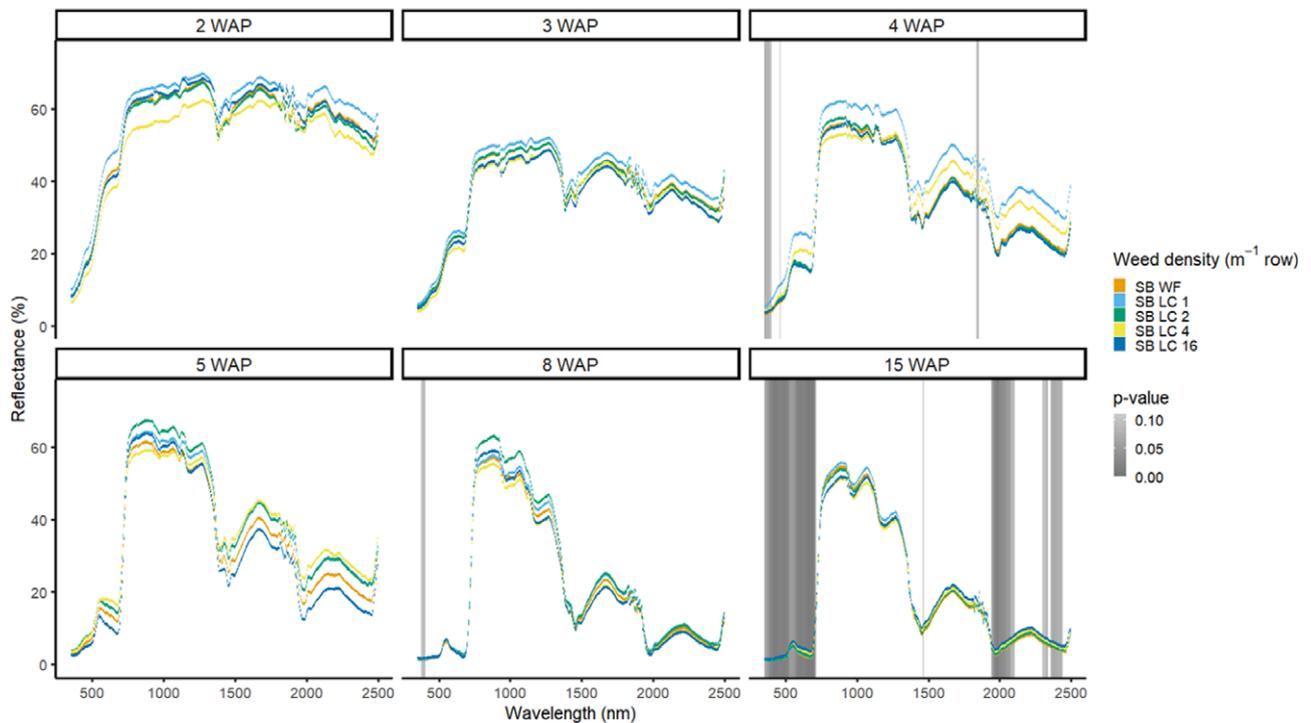


Figure 8. Spectral reflectance for all *Digitaria sanguinalis* (LC) densities with soybean (SB) and a weed-free control (SB WF), grouped by weeks after planting (WAP), at the Horticultural Crops Research Station, Clinton, NC, 2017.

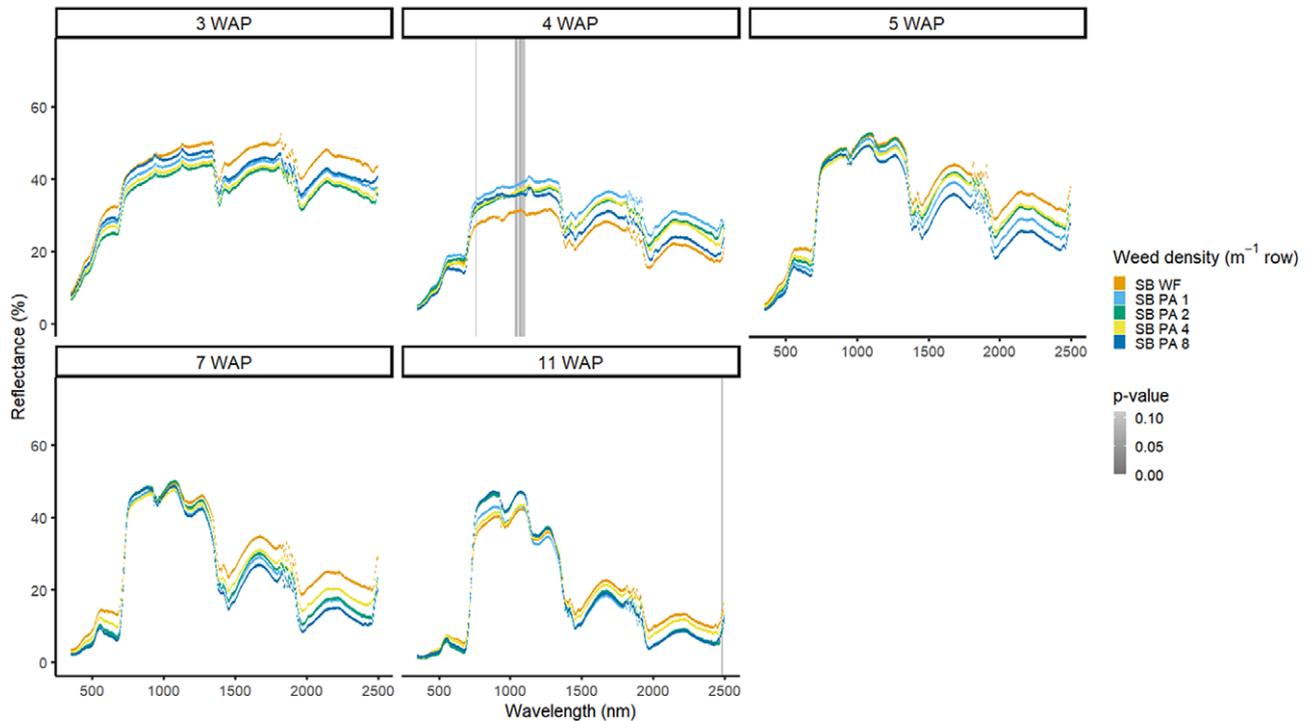


Figure 9. Spectral reflectance for all *Amaranthus palmeri* (PA) densities with soybean (SB) and a weed-free control (SB WF), grouped by weeks after planting (WAP), at the Horticultural Crops Research Station, Clinton, NC, 2016.

indicating that there were greater differences in plant biomass during this period when compared with the bare-ground plots. By 8 and 15 WAP, VIS, SWIR1, and SWIR2 regions had greater absorption than bare-ground plots due to the presence of *D. sanguinalis* biomass. By 15 WAP, the magnitude between spectra decreased because of the onset of senescence in *D. sanguinalis* at this reading date.

Amaranthus palmeri detection occurred at a similar timing to that of *D. sanguinalis* for each year. In 2016, differences in *A. palmeri* density were only noted at 5 WAP (VIS and SWIR2) and 11 WAP (VIS, SWIR1, and SWIR2) (Figure 14). In 2017, differences occurred earlier in the season (3 WAP) and across more spectra (Figure 15). Many of the differences between *A. palmeri* density were in the VIS, with late-season (8 and 15 WAP) differences occurring across most spectra in the SWIR1 and SWIR2 regions.

Weed and Crop Phenology

The phenology of crop and weed species was not affected by crop presence/absence and weed density, as phenological differences between densities were not noted (Table 2). Annual differences in phenological timing were different between years as defined by Meier (2018). At the earliest data-collection dates across years and cropping systems, weed species and density did not have established canopies and could not be differentiated from either weed-free or bare-ground treatments. Rapid establishment of weed species canopies, especially with *A. palmeri*, leads to increased absorption in VIS early in the season. Similarly, early detection of *D. sanguinalis* was due to increased canopy coverage of higher-density treatments. The establishment of weed canopy was critical to the differentiation between crop species and density.

Weed species phenology did not differ between densities, but overall weed biomass and height did change due to interspecific competition with each crop and intraspecific competition due to increased weed density (Basinger et al. 2019). Higher weed density tended to fill out a canopy more quickly, but individual plants tended to be smaller overall than at lower weed densities. Lower-density weed species toward the end of the season in each year tended to have greater canopy density per plant but were similar overall at higher plant densities. Higher densities of weed species tended to have greater absorption in the VIS, SWIR1, and SWIR2 earlier in the season. As canopies of lower-density treatments became established, differences between weed densities were observed at fewer SWIR1 and SWIR2 spectra but continued in the VIS region. Plant phenology is important for the differentiation and detection of weed species. Phenology is not often considered in studies using remote sensing for species discrimination (Santos et al. 2016; Schmidt and Skidmore 2003), but spectral reflectance does change with changing crop phenology (Basinger et al. 2020; Vina et al. 2004). Phenology and biophysical characteristics are inherently linked with one another and are correlated with hyperspectral reflectance (Lausch et al. 2015). Changing plant phenology can have effects on internal leaf structure and chlorophyll concentration, which could explain some of the overall changes in spectra during each growing season (Poorter et al. 2009; Slaton et al. 2001). High temporal data collection in the early season is important for the detection of weed species, as plant phenology changes rapidly during this time. Early-season detection is also important in agricultural systems, because weed management strategies are most effective when weeds are small.

This study aimed to elucidate spectral changes in a soybean crop with varying densities of two weed species across several

Table 2. Weeks after planting (WAP), date when spectral measurements were taken, growing degree days (GDD), mean plant phenology, and mean plant height across treatments in soybean in 2016 and 2017 at Horticultural Crops Research Station, Clinton, NC.

WAP	Date	GDD ^b	Plant phenology ^a			Plant height		
			Soybean	<i>Digitaria sanguinalis</i>	<i>Amaranthus palmeri</i>	Soybean	<i>Digitaria sanguinalis</i>	<i>Amaranthus palmeri</i>
2016								
0 WAP	June 6, 2016	0	Seed	Seed	Seed	0	0	0
3 WAP	June 29, 2016	358	Trifoliolate leaf on the second node unfolded	2 true leaves	4 true leaves	8	3	5
4 WAP	July 7, 2016	503	Trifoliolate leaf on the fifth node unfolded	Fifth true leaf expanded	2 side shoots visible	12	13	15
5 WAP	July 15, 2016	562	Trifoliolate leaf on the seventh node unfolded	Third internode visible	5 side shoots visible	20	28	40
7 WAP	July 28, 2016	801	First flowers opened	First awns visible	Full flowering: 50% of flowers open, first petals may have fallen	26	40	75
11 WAP	August 26, 2016	819	End of flowering with pods visible	Caryopsis watery ripe	Nearly all fruits have reached final size	48	82	140
2017								
0 WAP	June 12, 2017	0	Seed	Seed	Seed	0	0	0
2 WAP	June 28, 2017	266	Trifoliolate on the second node unfolded	2 true leaves visible	4 true leaves visible	8	3	5
3 WAP	July 6, 2017	429	Trifoliolate on the third node unfolded	3 tillers visible	4 side shoots visible	13	13	15
4 WAP	July 13, 2017	551	Third side shoot of first order visible	Fifth internode extended	9 or more extended internodes	20	28	40
5 WAP	July 19, 2017	671	First flower buds visible	Flag sheath just visibly swollen	First individual flowers visible, but still closed	26	53	75
8 WAP	August 10, 2017	1,038	About 60% of flowers open	Full flowering: 50% of flowers open	Nearly all fruits have reached final size	49	82	143
15 WAP	September 13, 2017	1,536	About 30% of pods have reached final length (15–20 mm)	Beginning of ripening or seed coloration/desiccation; fruit fully ripe	Seeds are fully ripe/beginning of leaf fall	90	83	143

^aPlant phenology based on the BBCH scale (Meier 2018).

^bGrowing Degree Day (GDD) calculated using a base temperature of 10 C.

phenological time points. The study showed that spectral reflectance is dynamic throughout the season and between seasons, with the magnitude and shape of each spectrum changing in some parts of the measured spectra, while other spectra were similar across observation dates despite differences in treatments (bare ground, weed-free soybean, density of *D. sanguinalis* or *A. palmeri*). These variations in absorbance, reflectance, and magnitude changes over the season could be used as targets to assess plant phenology and/or plant density. Spectral changes were seen more when there were differences in the amount of vegetation present, indicating that areas of greater weed populations could be spectrally distinguished from lower-density areas of the same species. Furthermore, plants that are more phenologically separate in terms of biomass production and reproduction and overall canopy structure can provide opportunities for spectral differentiation.

Given that much of the spectral differentiation in this study was in the VIS region, there is a significant opportunity to leverage these differences using standard RGB photographs coupled with machine learning to assist in the differentiation of crop and weed species. However, there are several spectral in the

SWIR1 and SWIR2 regions that could be further utilized for differentiation of species and weed density. Leveraging these additional spectra may provide opportunities to further improve the discrimination of plant species using artificial intelligence. Furthermore, understanding the impact of variations in magnitude of absorbance and reflectance of certain spectra as plant phenology changes could provide additional insight into building more accurate algorithms and sensor platforms for use in agricultural systems.

In this study, plant phenology was important in the detection and differentiation of weed species, and it should be incorporated in future studies. Additional challenges of identification of site-specific weed management include the development of an appropriate threshold to determine when weed management is necessary and the methods to be used to control weeds. This study focused on the detection and differentiation of individual weed species that were not part of a weed species complex but utilized reflectance spectra to differentiate between crops and weeds. The weed species complex should be considered, as this situation more accurately reflects the natural occurrence of weeds in agricultural systems.

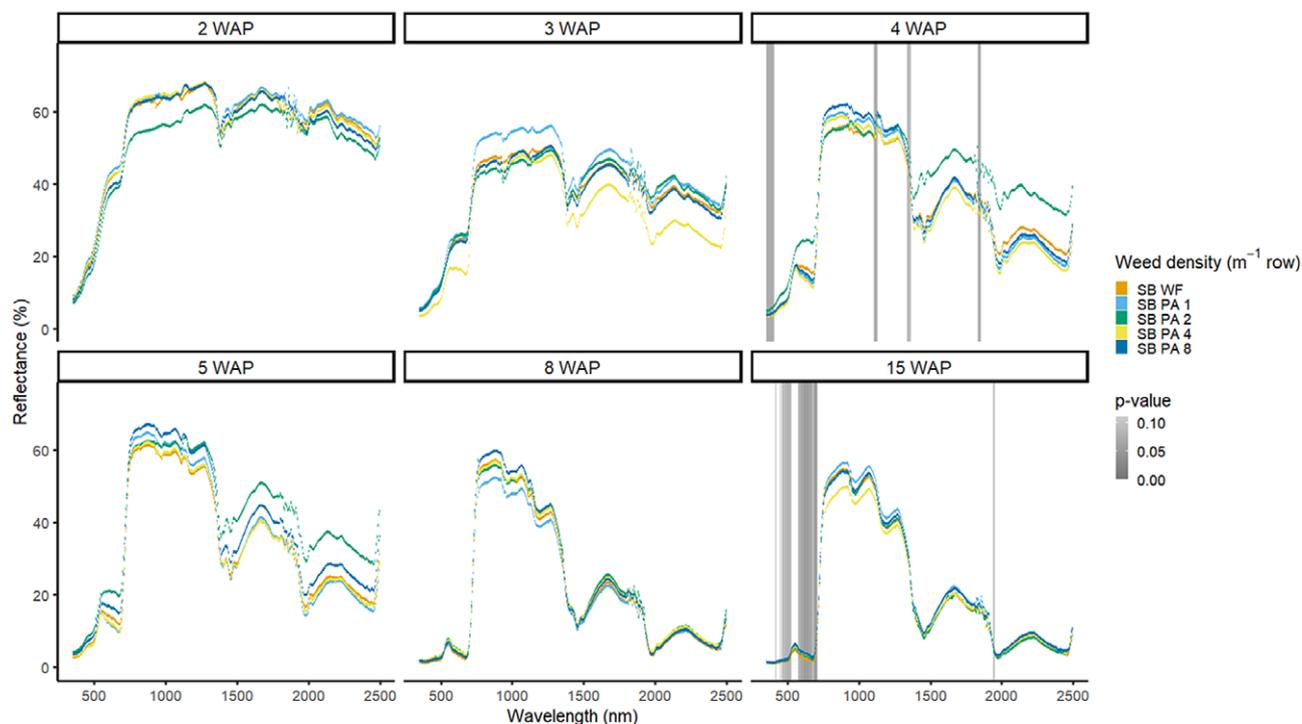


Figure 10. Spectral reflectance for all *Amaranthus palmeri* (PA) densities with soybean (SB) and a weed-free control (SB WF), grouped by weeks after planting (WAP), at the Horticultural Crops Research Station, Clinton, NC, 2017.

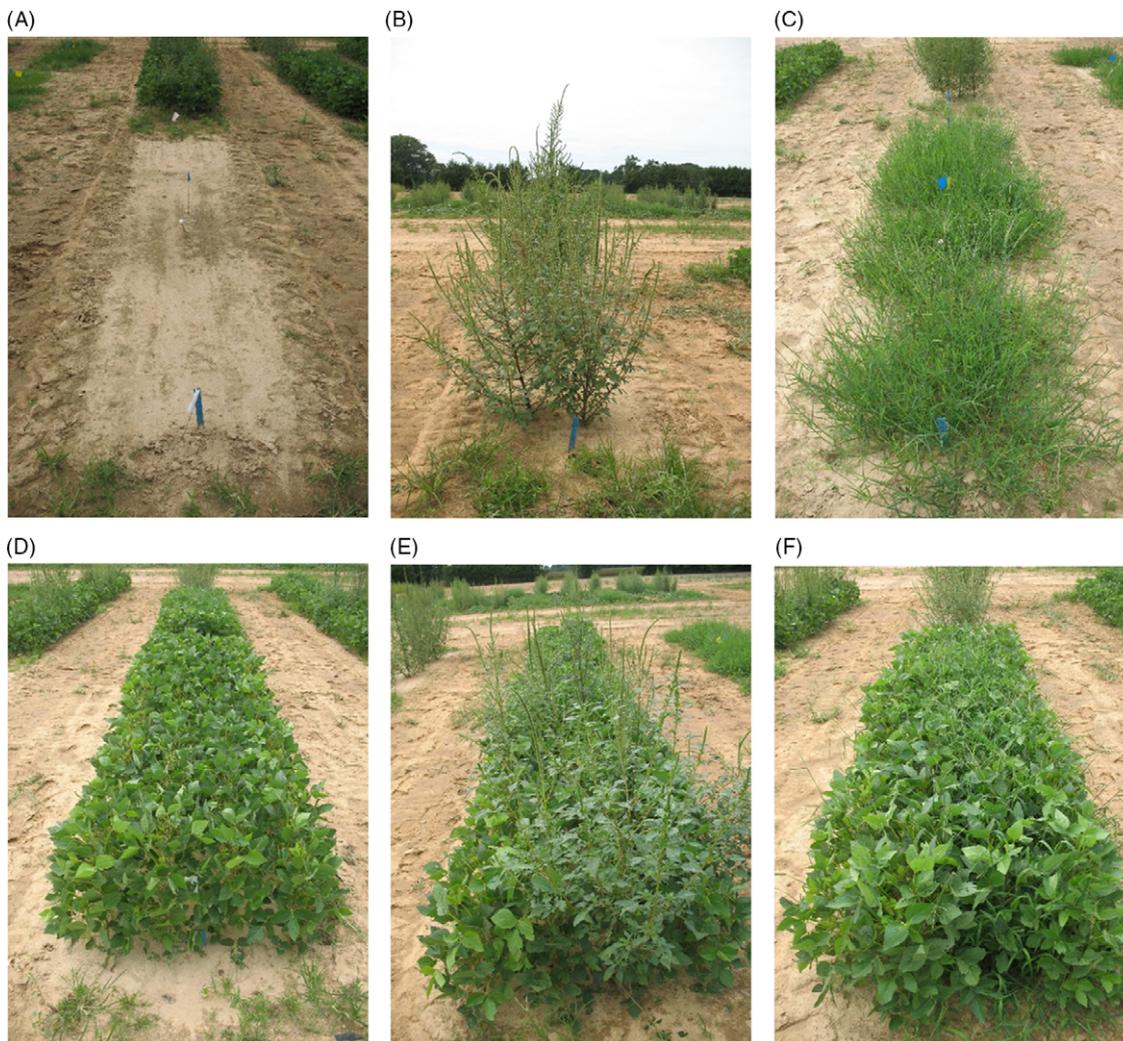


Figure 11. Visual representation of plant structure and growth of weeds with and without soybean: (A) bare ground, (B) *Amaranthus palmeri*, (C) *Digitaria sanguinalis*, (D) soybean, (E) soybean and *A. palmeri*, (F) soybean and *D. sanguinalis*. Any pictures with weeds represent the highest weed density of each species tested.

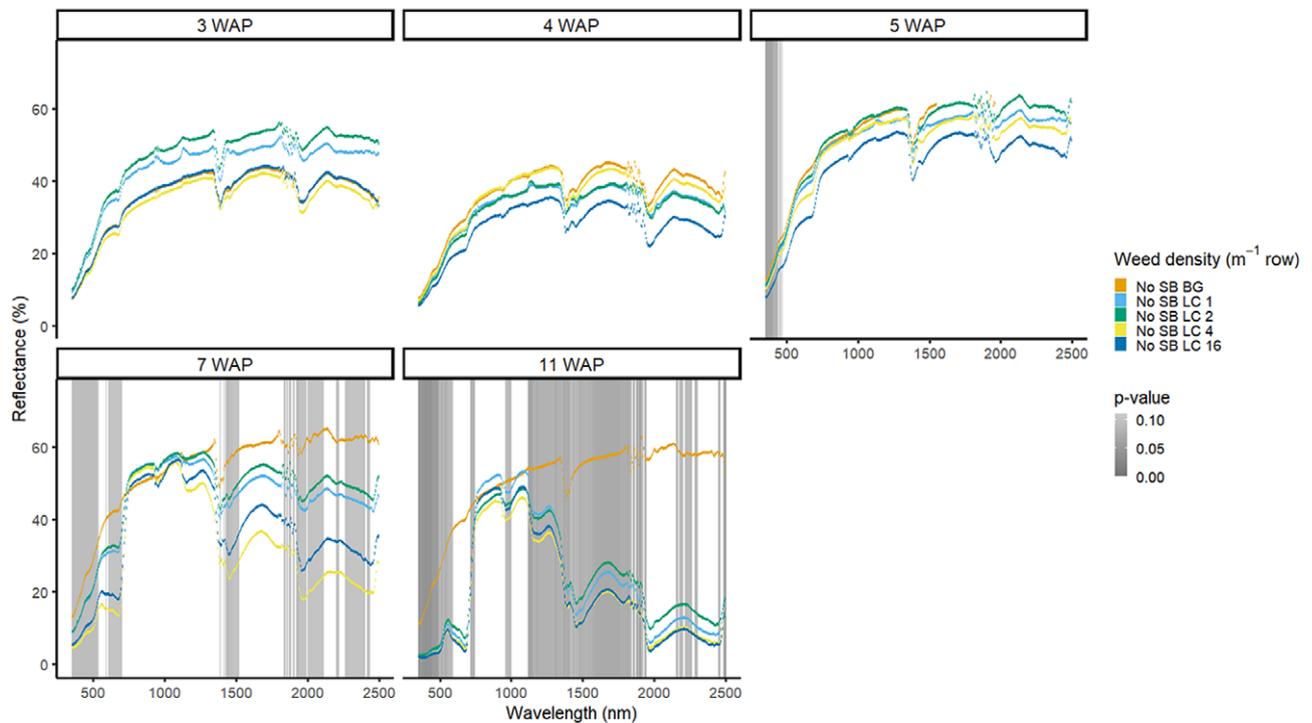


Figure 12. Spectral reflectance for all *Digitaria sanguinalis* (LC) densities without soybean (No SB) and a bare ground control (No SB BG), grouped by weeks after planting (WAP), at the Horticultural Crops Research Station, Clinton, NC, 2016.

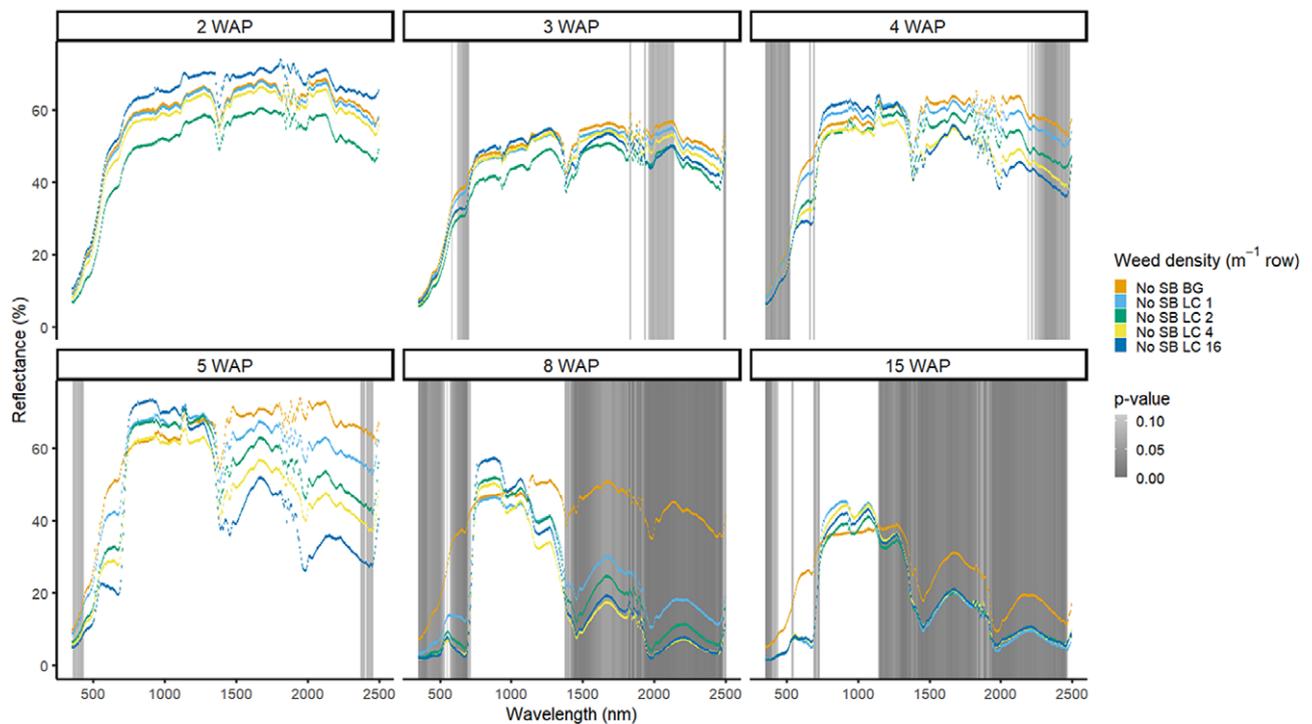


Figure 13. Spectral reflectance for all *Digitaria sanguinalis* (LC) densities without soybean (No SB) and a bare-ground control (No SB BG), grouped by weeks after planting (WAP), at the Horticultural Crops Research Station, Clinton, NC, 2017.

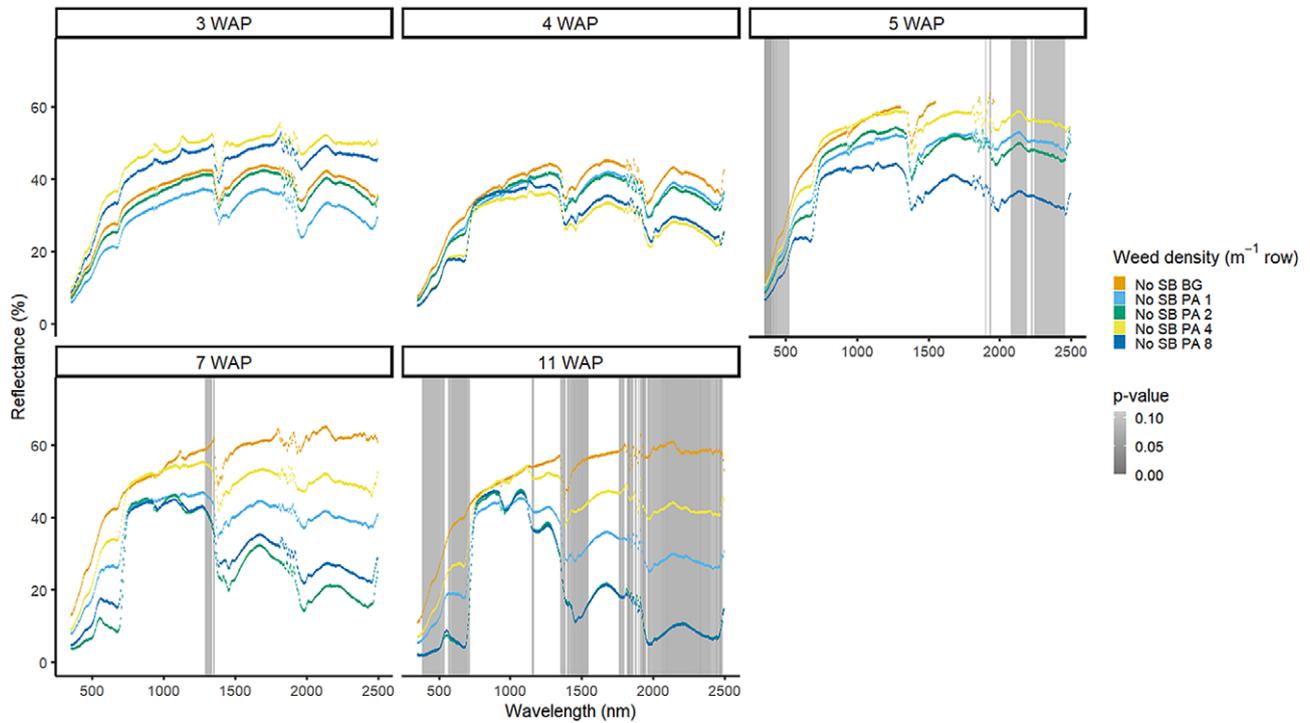


Figure 14. Spectral reflectance for all *Amaranthus palmeri* (PA) densities without soybean (No SB) and a bare-ground control (No SB BG), grouped by weeks after planting (WAP), at the Horticultural Crops Research Station, Clinton, NC, 2016.

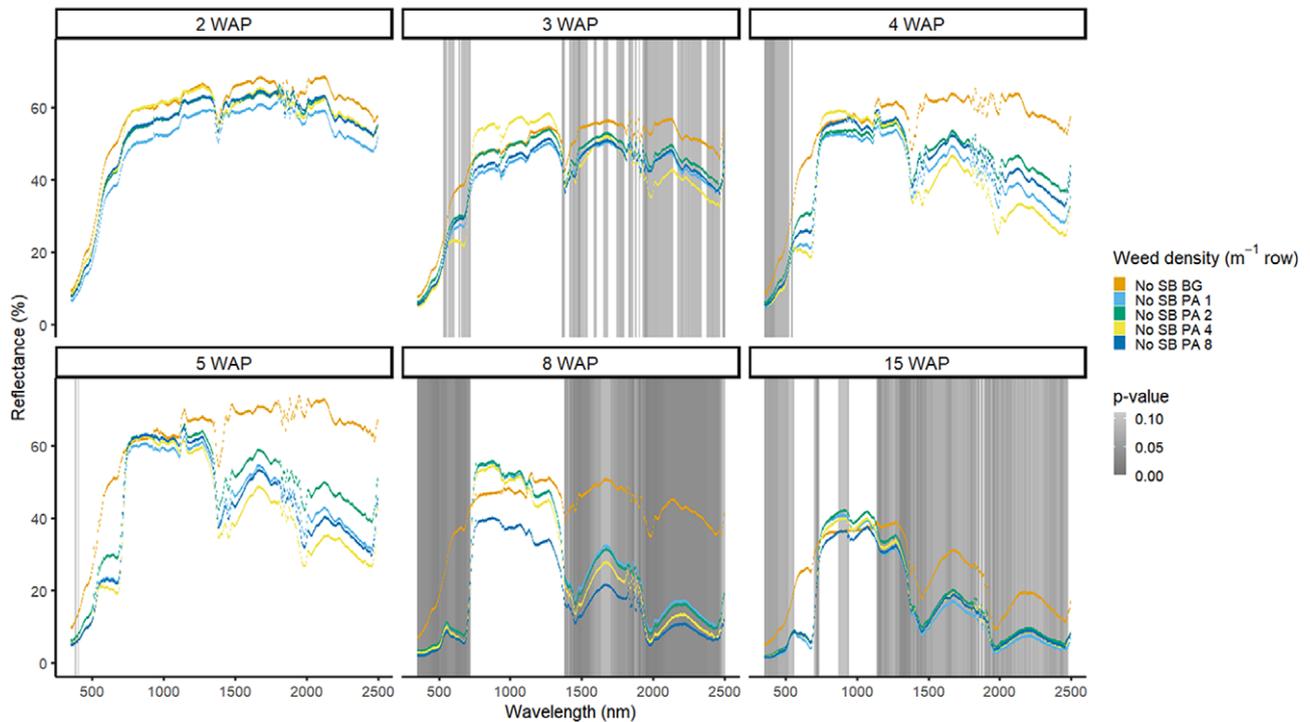


Figure 15. Spectral reflectance for all *Amaranthus palmeri* (PA) densities without soybean (No SB) and a bare-ground control (No SB BG), grouped by weeks after planting (WAP), at the Horticultural Crops Research Station, Clinton, NC, 2017.

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