Phenology affects differentiation of crop and weed species using hyperspectral remote sensing

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Abstract

The effect of plant phenology and canopy structure of four crops and four weed species on reflectance spectra were evaluated in 2016 and 2017 using in situ spectroscopy. Leaf-level and canopy-level reflectance were collected at multiple phenologic time points in each growing season. Reflectance values at 2 wk after planting (WAP) in both years indicated strong spectral differences between species across the visible (VIS; 350–700 nm), near-infrared (NIR; 701–1,300 nm), shortwave-infrared I (SWIR1; 1,301–1,900 nm), and shortwave-infrared II (SWIR2; 1,901–2,500 nm) regions. Results from this study indicate that plant spectral reflectance changes with plant phenology and is influenced by plant biophysical characteristics. Canopy-level differences were detected in both years across all dates except for 1 WAP in 2017. Species with similar canopy types (e.g., broadleaf prostrate, broadleaf erect, or grass/sedge) were more readily discriminated from species with different canopy types. Asynchronous phenology between species also resulted in spectral differences between species. SWIR1 and SWIR2 wavelengths are often not included in multispectral sensors but should be considered for species differentiation. Results from this research indicate that wavelengths in SWIR1 and SWIR2 in conjunction with VIS and NIR reflectance can provide differentiation across plant phenologies and, therefore should be considered for use in future sensor technologies for species differentiation.

Introduction

Remote sensing in agriculture has continued to expand as pressure on farmers to produce crops more efficiently has increased. For profitable farming, farmers are exploring diverse cropping systems, resulting in a greater variety of weed control challenges. Integrating remote-sensing technology into agriculture to improve efficiency and inform management decisions has been a topic of research for decades (Lamb and Brown 2001). With improvements in sensor technology, data management, storage, and processing power, a resurgence of interest in agricultural remote sensing has occurred in the past 15 years (Hung et al. 2014; Hunt et al. 2014; López-Granados 2011). Remote sensing has been used to estimate crop yield and biomass (Bandyopadhyay et al. 2014; Casanova et al. 1998; Diker and Bausch 2003; Hansen and Schjoerring 2003; Ray et al. 2006), water stress (Bandyopadhyay et al. 2014; Penuelas et al. 1993), crop nutrient status (Cohen et al. 2010; Goel et al. 2003; Jain et al. 2007), herbicide injury (Everman et al. 2008; Henry et al. 2004a), damage caused by plant diseases and insects (Del Fiore et al. 2010; Mahlein et al. 2013), and the detection and control of weeds (Bolch et al. 2020; Burks et al. 2002, 2005; Henry et al. 2004b; Huang et al. 2016; López-Granados 2011; Medlin et al. 2000). Despite a growing body of research in which remote sensing in agriculture was used, additional research is needed to continue to narrow the gap between data collection and practical management decisions.

One application of remote sensing in agriculture is the site-specific management of weeds (Jurado-Expósito et al. 2003; López-Granados 2011). Site-specific weed management can reduce the need for broadcast applications, limiting potential environmental impacts of herbicide use, tillage, soil compaction, off-target pesticide movement, and lowering overall input costs. Site-specific weed management requires accurate differentiation between crop and weed species to be able to control weed species while leaving crops untouched. Successful differentiation of plant species can be challenging because many weed and crop species share similar biophysical plant traits. Species-level discrimination using remote sensing has been successful using plant

Table 1. Crop and weed species used for plant spectral analysis at the Horticulture Field Laboratory, Raleigh, NC, in 2016 and 2017.

Crop/weed	Common name	Scientific name	Source
Crop	Sweetpotato	*Ipomoea batatas* L. Lam. 'Covington'	Jones Family Farm, Bailey, NC
Crop	Peanut	*Arachis hypogaea* L. 'Bailey'	NC Foundation Seed Producers, Zebulon, NC
Crop	Soybean	*Glycine max* L. Merr. 'AG6535'	Asgrow Seed, St. Louis, MO
Crop	Cucumber	*Cucumis sativus* L. 'Arabian'	Seminis Vegetable Seeds, St. Louis, MO
Weed	Yellow nutsedge	*Cyperus esculentus* L.	Azlin Seed Service, Leland, MS
Weed	Large crabgrass	*Digitaria sanguinalis* (L.) Scop.	Azlin Seed Service
Weed	Palmer amaranth	*Amaranthus palmeri* S. Watson	Collected field accession, Horticulture Crops Research Station, Clinton, NC
Weed	Common ragweed	*Ambrosia artimisiifolia* L.	Collected field accession, private grower field, Moyock, NC

biophysical traits such as leaf morphology, canopy structure, and plant biochemistry (Asner 1998; Gamon et al. 1997). More recent approaches have used complex machine-learning algorithms that use combinations of standard color photographs and multispectral images to successfully separate weed species from a planted crop (Dian Bah et al. 2018; Farooq et al. 2019). Even with these approaches using color and multispectral images, other approaches may be necessary to further improve the reliability for crop and weed species to be distinguished from one another.

One approach for making the distinction between each of these species has focused on reflectance spectra, or the wavelengths of light reflected off the plant canopy (Gray et al. 2009; Hemming and Rath 2001; Henry et al. 2004a; Henry et al. 2004b). These spectra can be collected through proximal sensors mounted on tractors or other vehicles, handheld proximal sensors, or via satellites or unmanned aerial vehicles (Thorp and Tian 2004). Hyperspectral remote sensing, or spectroscopy, provides a high degree of spectral resolution, capturing solar radiation reflected off plant surfaces in narrow wavelength bands collected between 350 and 2,500 nm (Jensen 2007). With the human eye and color photographs, the spectra detected are limited to visible light (VIS; 300–700 nm). However, wavelengths outside of the visible range such as the near-infrared (NIR; 701–1,300 nm), shortwave-infrared I (SWIR1; 1,301–1,900 nm), and shortwave-infrared II (SWIR2; 1,901–2,500 nm) may improve species differentiation by detecting differences unnoticed with the naked eye, with common photographic equipment, or even multispectral cameras (Ustin et al. 2004). Remote sensing using spectroscopy has been used to discriminate between species in agricultural (Henry et al. 2004b; Koger et al. 2004) and nonagricultural settings (Santos et al. 2012; Schmidt and Skidmore 2003). Although previous research has been able to differentiate species in the VIS and NIR range (Cho et al. 2010; López-Granados 2011), newer sensors can detect bands in the SWIR (1,300–2,500). SWIR spectra may provide additional information for the refinement of species differentiation algorithms, provide new spectra to be included in multispectral sensors, or provide additional utility of remote sensing in agriculture.

Although researchers have successfully been able to discriminate between crop and weed species in field settings using multispectral and hyperspectral remote sensing, these studies have been limited to only a few crops and weed species (Gray et al. 2009; Hemming and Rath 2001; Henry et al. 2004a, 2004b; Koger et al. 2004). These species include common cocklebur (*Xanthium strumarium* L.), sicklepod [*Senna obtusifolia* (L.) Irwin & Barneby], pitted morningglory (*Ipomoea lacunosa* L.), and horsenettle (*Solanum carolinense* L.) in soybean [*Glycine max* (L.) Merr.] (Henry et al. 2004a; Henry et al. 2004b; Koger et al. 2004; Medlin et al. 2000); Palmer amaranth (*Amaranthus palmeri* S. Watson) in cotton (*Gossypium hirsutum* L.); climbing milkweed

[*Funastrum cynanchoides* (Decne.) Schltr.] in orange [*Citrus × sinensis* (L.) Osbeck.]; ragweed parthenium (*Parthenium hysterophorus* L.) in carrot (*Daucus carota* L. var. *sativus* Hoffm.); London rocket (*Sisymbrium irio* L.) in cabbage (*Brassica oleracea* var. *capitata*); and johnsongrass [*Sorghum halepense* (L.) Pers.] in sorghum [*Sorghum bicolor* (L.) Moench ssp. *bicolor*] (Menges et al. 1985). Although these studies address some problematic weed species in crops, the list of these species is not exhaustive and more research is needed. Furthermore, a previous study indicated that phenologic changes during a growing season can affect species differentiation (López-Granados et al. 2008) and can be used to increase weed detection accuracy from hyperspectral remote-sensing data (Andrew and Ustin 2008; Glenn et al. 2005; Lass and Callihan 1997). However, limited research exists on optimal timing for species differentiation in cropping systems. Thus, the objectives of this study were to (1) determine if four common crops and four problematic weed species can be differentiated on the basis of their hyperspectral reflectance, (2) identify spectral regions that allow for species differentiation, (3) evaluate the effect of phenologic stage of crops and weeds on crop and weed differentiation, and (4) determine the impact of canopy structure on spectral reflectance using canopy- and leaf-level spectra.

Materials and Methods

Plant Potting

This study was conducted in 2016 and 2017 at North Carolina State University's Horticulture Field Laboratory, Raleigh, NC (35°47′N, 78°41′ W). Plants representing most common or troublesome weeds in agronomic and horticultural crops [(cucumber (*Cucumis sativus* L.), peanut (*Arachis hypogea* L.), soybean, sweetpotato [*Ipomoea batatas* (L.) Lamm.], and weed species (Palmer amaranth, common ragweed (*Ambrosia artimisiifolia* L.), yellow nutsedge (*Cyperus esculentus* L.), and large crabgrass (*Digitaria sanguinalis* (L.) Scop.] (Webster 2010, 2014) were planted into 95-L pots (Table 1) on May 27, 2016, and May 24, 2017. Pots were watered for the first week after planting (WAP) to allow for seed germination and transplant establishment. After emergence, plants were thinned to a single plant pot^{-1}. Sweetpotato was the only plant species that was not seeded; it was transplanted using nonrooted cuttings with 1 to 2 leaves.

Each pot contained two layers: the bottom layer contained 28 L of a soilless potting medium (Jolly Gardener 4P Growing Mix; Oldcastle Lawn and Garden, Poland Spring, ME), and the upper layer contained 38 L of native Norfolk loamy sand (Fine-loamy, kaolinitic, thermic Typic Kandiudults), with a humic matter of 0.41% and soil pH 6.0, collected from the Horticultural Crops Research Station near Clinton, NC (35°1′N, 78°16′W).

The purpose of the soilless medium was to draw water from a water-filled tray while minimizing disturbance of the upper-field soil layer. The field soil was used to mimic soil reflectance that would be present in an agricultural field.

Pots were arranged in a randomized complete block design with four replications and placed in a large, polyethylene-lined, 1.2 × 2.4 m trays 76-mm tall to allow pots to be watered from the bottom. To ensure plants did not experience water stress, trays were filled with water twice daily (morning and evening). Each pot was fertilized every 14 d with 24-8-16 (N-P-K; Miracle-Gro; Miracle-Gro Lawn Products, Marysville, OH) at a rate of 270 kg ha^{-1}. The study was terminated 10 WAP.

Spectral Data Collection

Five measurements for each pot were taken using a portable field spectrometer equipped with a handheld foreoptic with a 44-rad instantaneous field of view (PSM-2500; Spectral Evolution, Lawrence, MA). Each canopy measurement was collected weekly when weather was appropriate from nadir at 1 m above the plant canopy, resulting in a ground instantaneous field of view (GIFOV) of 0.44 m^{-2}. Measurements were taken at full-intensity sunlight (10:00–14:00 hours). The spectrometer was calibrated using a white reflectance panel (Spectralon; LabsSphere, North Sutton, NH) approximately every 15 min to account for variations in sun angle and atmospheric conditions. Pots containing no plant material were used as a soil reference to ensure plants could be differentiated from the soil.

In addition to canopy-level spectra, leaf-level spectra from the upper and the lower plant canopy were collected immediately after canopy-level measurements using a leaf clip attachment (ILM-105; Spectral Evolution). The leaf clip used an independent light source containing a 5-W tungsten halogen bulb delivered to the leaf clip by fiber-optic cable to minimize the plant tissue degradation due to heat from the light source. The leaf clip allowed for additional data collection during times when canopy level data could not be obtained and for quantification of contributions of each portion of the canopy to the overall plant reflectance. Upper leaf measurements were taken on the newest unfurled leaf on each species. Data collected on species with compound leaves were taken from multiple leaflets on the uppermost leaf. Lower leaf measurements were taken on the most mature true leaves in the plant canopy.

Measurement of Plant Biological Characteristics

Plant height, leaf angle, phenologic stage, and soil moisture content were measured on each data collection date. Leaf angle was measured using a protractor with a weight attached to a light-weight string. Five leaves on each plant were measured to determine the mean leaf angle. Measurements were taken from the horizontal position, where 0° was perpendicular to the soil surface. Angles greater than 0° (i.e., more erect leaf types) were measured in positive degrees, whereas leaf angles less than 0° (i.e., leaf blade angled downward toward the soil surface) were given negative values. The phenologic stage was assessed using methods described by Meier (2001). Soil-moisture sensors (Watermark soil moisture sensor, model 200SS; The Irrometer Company, Riverside, CA) were inserted 0.15-m deep in two of four replications to monitor soil-water availability in the pots and to ensure plants were not water stressed during spectral measurements. Soil moisture sensors were not placed in all replications, because of the high cost of each sensor.

Table 2. Average Monthly Temperature and Growing Degree-Days (base, 10 C) for Horticulture Field Laboratory, Raleigh, NC, from May to August for 2016 and 2017.

Month	Temperature 2016	Temperature 2017	Growing degree-day 2016	Growing degree-day 2017
	——C——		——Base 10 C——	
May	19.2	20.2	293	327
June	24.4	23.6	439	414
July	26.7	26.6	537	529
August	26.6	25.2	537	487

To assess environmental conditions and account for variability between years, daily mean temperature, precipitation, and growing degree-days were obtained from the North Carolina Climate Office for the nearest weather station at the NC State University Lake Wheeler Road Field Laboratory (35°43′N, 78°40′W) located approximately 6.5 km southeast of the study location. Weather at the study site was consistent across years (Table 2).

Data Analysis

All spectral reflectance data (350–2,500 nm) for each plot were graphed by date. Data quality was visually assessed to remove errant or noisy measurements caused by human error or environmental interference. Errant readings were noted during field measurements for removal. The mean spectral reflectance for each species for each date was calculated on the remaining spectral measurements. To examine spectral separation between all species, a nonparametric Kruskal-Wallis test on ranks (P ≤ 0.1) (Corder and Foreman 2009) by date, wavelength, and spectral type (i.e., canopy, uppermost-leaf, lowermost-leaf) was used. To further test for differences between individual species, a Mann-Whitney U (P ≤ 0.1) test was used for all possible pairwise species comparisons, and species were considered to be "differentiable" at these spectra when resulting P-values were less than 0.1. Both tests are nonparametric tests that do not assume the normal distribution of data. These tests use median values for data and can account for unequal numbers of species samples. Because there were many species comparisons for canopy level, upper leaf, and lower leaf, results where differences across multiple spectra and date were observed are discussed. Additional data are included as supplemental data or can be accessed in the report of Basinger (2018). All statistical analyses and data visualization were performed in R, version 3.5.2 (R Foundation, Vienna, Austria).

Results and Discussion

Species Differentiation

In both years, species were differentiable on the basis of their canopy hyperspectral reflectance. However, spectral regions where species were differentiable at P ≤ 0.1 were not consistent across all reading dates (Figures 1 and 2). Several species, such as yellow nutsedge, soybean, and sweetpotato, were consistently differentiable from other species throughout the season (Supplementary Figures S1–S6). Other species, such as Palmer amaranth, cucumber, and ragweed, showed early-season differentiation but were not differentiable at readings later in the season (Figures 3 and 4; Supplementary Figures S7–S14). Soil-moisture measurements collected at each reading date indicated that soil moisture did not fall
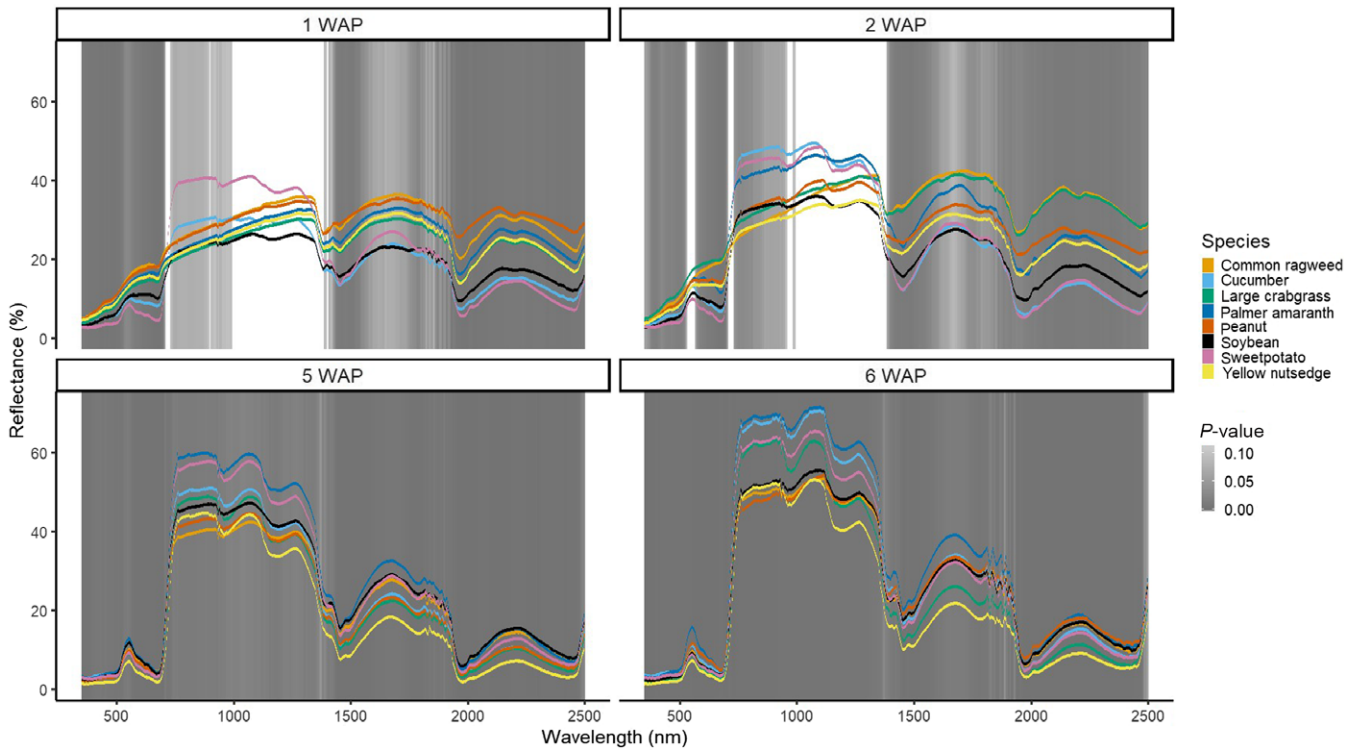
Figure 1. Canopy-level spectral reflectance curves for crop and weed species, Raleigh, NC, 2016. Species are indicated by color and reflectance spectra grouped by weeks after planting (WAP). Wavelengths at which species differences, as indicated by the Kruskal-Wallis at test P ≤ 0.1, are indicated as a continuous variable behind the spectral reflectance curves.
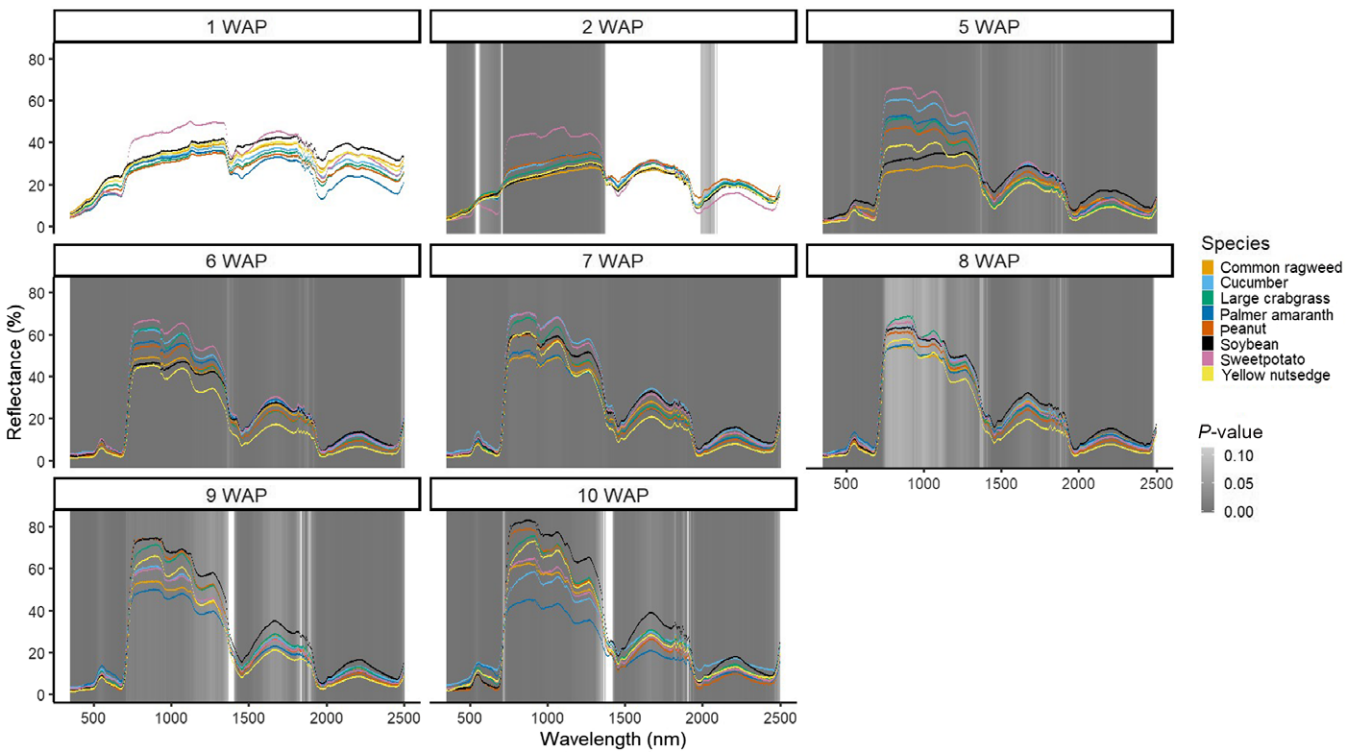

Figure 2. Canopy-level spectral reflectance curves for crop and weed species, Raleigh, NC, 2017. Species are indicated by color and reflectance spectra grouped by weeks after planting (WAP). Wavelengths at which species differences, as indicated by the Kruskal-Wallis at test P ≤ 0.1, are indicated as a continuous variable behind the spectral reflectance curves.
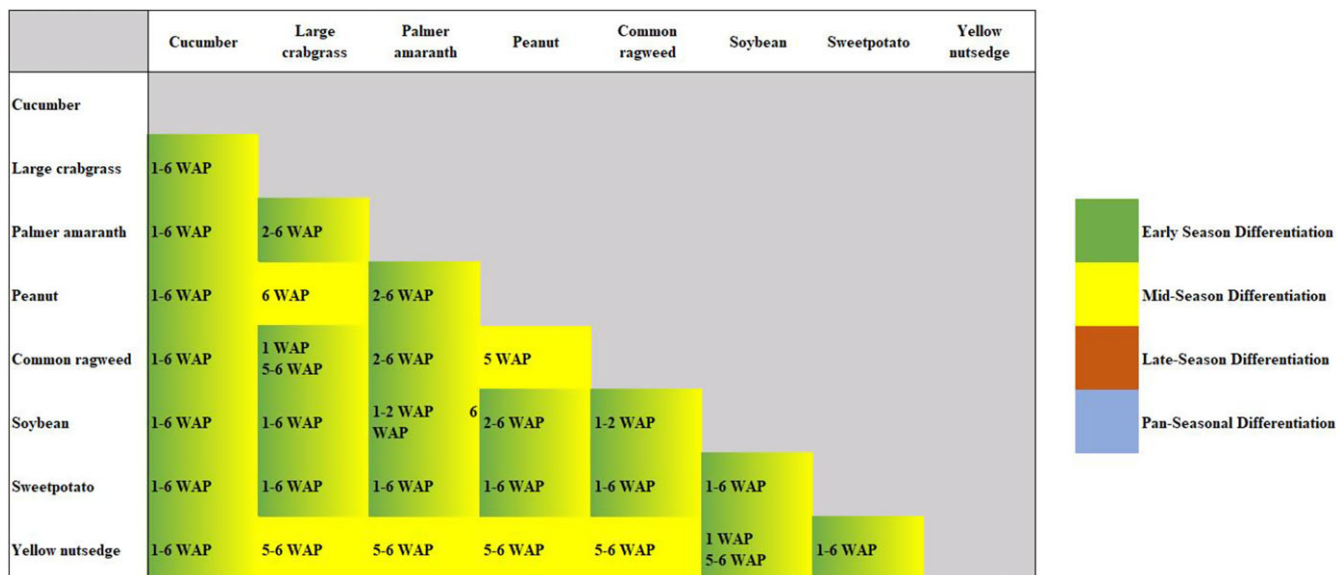
Figure 3. Weeks after planting (WAP) in 2016 at which species could be differentiated. Measurements were taken at weeks 1, 2, 5, and 6 WAP. Species were considered differentiable if ≥10 wavelengths were significant according to the Kruskal Wallis test at the P ≤ 0.1 level.
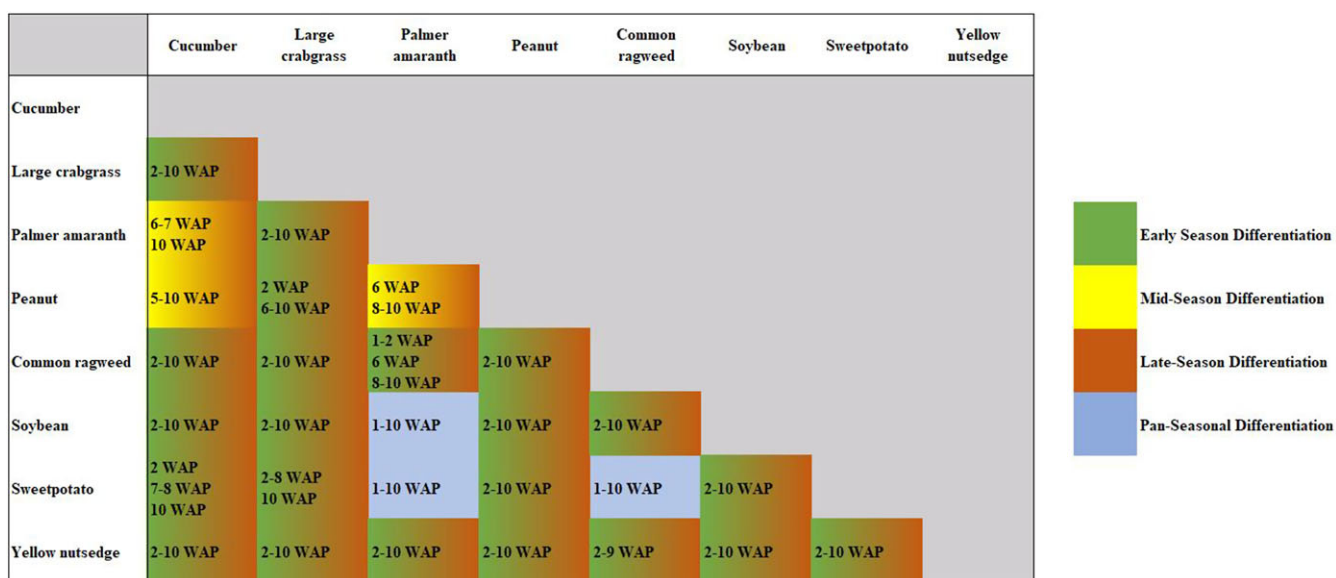
Figure 4. Weeks after planting (WAP) in 2017 at which species could be differentiated. Measurements were taken at weeks 1, 2, 5, 6, 7, 8, 9, and 10 WAP. Species were considered differentiable if ≥10 wavelengths were significant according to the Kruskal Wallis test at the P ≤ 0.1 level.

below field capacity. Plants did not present any signs of nutrient deficiencies, and senescence that occurred in this study was natural and occurred after reproduction. Species separation has been noted by others (Gray et al. 2009; Koger et al. 2004; Schmidt and Skidmore 2003) but has not addressed season-long changes in plant phenology with changes in spectral reflectance.

Plant Phenology

Spectral differences between species were detected as early as 1 WAP in 2016 and 2 WAP in 2017 at the canopy level but were not constant season-long with changing plant phenology (Figures 1–4). Plant species in this study at gross, distinctly different phenologic stages according to Meier (2001), were often

differentiable (Table 3). Therefore, plants with early-establishing canopies were more readily differentiated early in the season from species with more slowly establishing canopies. Early-season differentiation was seen for most species in the VIS and NIR, coinciding with the establishment of the plant canopy. The group-wise differences resulting from the Kruskal-Wallis test occurring early in the season were likely due to the quick canopy establishment of sweetpotato (transplanted), cucumber, and Palmer amaranth. Cucumber germinated (~4 d) and established a canopy (large cotyledons and large true leaves) more quickly than other species (Table 3). Palmer amaranth quickly established a canopy after germination, allowing for adequate leaf area for detection of the species compared with bare ground. Palmer amaranth's rapid canopy establishment has been documented by others

Table 3. Average Plant Phenology (BBCH Scale) With Weeks After Planting, Type of Spectral Measurement, Date, and Growing Degree-Days When Spectral Measurements Were Taken; Horticulture Field Laboratory, Raleigh, NC, from May to August for 2016 and 2017.

WAP[a]	Leaf- or canopy-level measurement	Date	GDD[b]	CR	CU	LC	PA	PN	SB	SP	YN
							Phenology[c]				
0	None	May 27, 2016	0	Seed	Seed	Seed	Seed	Seed	Seed	Nonrooted cutting	Tuber
1	Canopy	June 3, 2016	114	Cotyledons completely unfolded	Cotyledons completely unfolded	First true leaf emerged	Cotyledons completely unfolded	Cotyledons completely unfolded	Cotyledons completely unfolded	2 true leaves completely unfolded	First true leaf emerged
2	Canopy & leaf level	June 9, 2016	214	First leaf pair visible	Second true leaf on main stem unfolded	First true whorl unfolded	2 true leaves unfolded	Fourth true leaf unfolded	Trifoliate on the second node unfolded	5 true leaves completely unfolded	3 true leaves emerged
4	Leaf level	June 24, 2016	445	Fourth leaf pair visible	3 flower initial with elongate ovary visible on mainstem	5 node stage of stem elongation	≥9 visibly extended internodes on the main shoot	Seventh side shoot visible	Second side shoot of first-order visible	5 visibly extended stem internodes	Constant new development of young plants
5	Canopy & leaf level	July 1, 2016	564	5 visibly extended internodes	First flower on tertiary side shoot open	8 node stage of stem elongation	Inflorescence visible	Ninth side shoot visible	Sixth side shoot of first-order visible	Storage roots begin to develop	Constant new development of young plants
6	Canopy & leaf level eaf level	July 8, 2016	696	6 visibly extended internodes	Fourth fruit on main stem has reached typical size and form	Flag leaf sheath extending	First individual flowers visible (still closed)	Beginning of flowering	Seventh side shoot of first-order visible	Continued development of storage roots	Constant new development of young plants
7	Leaf level	July 16, 2016	849	8 visibly extended internodes	Sixth fruit on main stem has reached typical size and form	First awns visible	Full flowering: 50% of flowers open	Full flowering	Flower buds visible	Continued development of storage roots	Constant new development of young plants
							2017				
0	None	May 24, 2017	0	Seed	Seed	Seed	Seed	Seed	Seed	Nonrooted cutting	Tuber
1	Canopy & leaf level	June 3, 2017	140	Cotyledons completely unfolded	Cotyledons completely unfolded	First true whorl unfolded	First true leaves unfolded	First true leaf unfolded	Hypocotyl with cotyledons breaking through soil surface	2 true leaves completely unfolded	Shoot breaks through soil surface
2	Canopy & leaf level	June 9, 2017	221	First true leaf unfolded	Second true leaf on main stem unfolded	3 whorls unfolded	Second side shoot visible	Second true leaf unfolded	Emergence: hypocotyl with cotyledons emerged above soil surface ("cracking stage")	First side shoot visible	≥9 true leaves visible
4	Leaf level	June 22, 2017	422	3 true leaves unfolded	Second flower on initial with elongate ovary visible on main stem	2 node stage of stem elongation	≥9 visibly extended internodes	≥9 sides shoots visible	Trifoliate on 2nd node unfolded	≥9 visibly extended stem internodes	First young plants visible
5	Canopy & leaf level	June 29, 2017	516	6 side shoots visible	Tenth flower open on main stem	≥9 visibly extended stem internodes	Inflorescence visible	First flower petals visible	Trifoliate on sixth node unfolded	Storage roots begin to develop	Constant new development of young plants
6	Canopy & leaf level	July 7, 2017	649	≥9 side shoots visible	Third fruit on a tertiary side shoot has reached typical size and form	Flag leaf sheath just visibly swollen	First flowers opening	Continuation of flowering	Fourth side shoot of first-order visible	Continued development of storage roots	Constant new development of young plants
7	Canopy & leaf level	July 14, 2017	774	≥9 side shoots visible	Fourth fruit on a tertiary side shoot has reached typical size and form	Flag sheath opening	Full flowering: 50% of flowers open	Full flowering	Flower buds visible	Continued development of storage roots	Constant new development of young plants
8	Canopy & leaf level	July 20, 2017	1047	≥9 side shoots visible	30% of fruits show typical fully ripe color	Half of the inflorescence emerged	End of flowering	First carpophore pegs penetrating the soil	First flower petals visible; flower buds still closed	Continued development of storage roots	Constant new development of young plants
9	Canopy & leaf level	July 30, 2017	1120	≥9 side shoots visible	60% of fruits size typical, fully ripe color	Beginning of fruit ripening and coloration	Beginning of fruit ripening and coloration	End of flowering	About 20% of flowers open	Swelling of storage roots; top of roots above soil surface	Constant new development of young plants
10	Canopy & leaf level	August 4, 2017	1864	≥9 side shoots visible	Fruit are fully ripe, plant in decline	Fruit fully ripe	Fruit continuing to ripen, but not fully ripe, plant beginning to senesce	Beginning of pod development	Full flowering: ~50% of flowers open	Some storage roots have reached harvestable size	Constant new development of young plants

[a]Abbreviations: CR, common ragweed; CU, cucumber; LC, large crabgrass; PA, Palmer amaranth; PN, peanut; SB, soybean; SP, sweetpotato; WAP, weeks after planting; YN, yellow nutsedge.
[b]Cumulative growing degree-day calculated using a base temperature of 10 C from the day of crop and weed planting.
[c]Phenology determined using method of Meier (2001).

(Ehleringer 1983; Horak and Loughin 2006). Rapid canopy establishment resulted in higher absorbance in the spectra associated with chlorophyll *a* and *b* at 400 to 460 nm and 660 to 685 nm, respectively (Blackburn 2007). Canopy establishment is also associated with greater NIR reflectance as a result of increased scattering in the leaf spongy mesophyll as leaf area increases (Asner 1998; Jensen 2007), as seen in Figures 1 and 2.

Seasonal phenologic changes are often associated with the accumulation of biomass, reproduction, and senescence. Cucumber and Palmer amaranth spectra showed an increase in reflectance during flowering in the VIS around 550 nm at 7 WAP (2017) for cucumber and 6 WAP (2016) and 8 WAP (2017) for Palmer amaranth compared with other species in the study (Figures 1 and 2). Changes in spectra due to phenology have been observed for weeds in other systems as well. Andrew and Ustin (2009) highlighted the small white flowers of perennial pepperweed (*Lepidium latifolium* L.), an invasive herb pervasive in the West and Midwest, as important for detecting spectral differences between it and other species in the landscape.

In the early season, differentiation of plant reflectance spectra was often tied to the VIS and NIR (Figures 1 and 2). Spectral differences between species after canopy establishment, in the latter part of the season, shift from the NIR to the VIS, SWIR1, and SWIR2 regions. Others have reported that SWIR spectral regions are associated with key plant structural components such as lignin, starch, and cellulose, often tied to plant maturation (Curran 1989; Kokaly et al. 2009; Thenkabail et al. 2004). When some species were at maturation, such as Palmer amaranth, cucumber, and sweetpotato, other species such as peanut, yellow nutsedge, and soybean were still in a vegetative or reproductive stage. Furthermore, at soybean flowering, sweetpotato, Palmer amaranth, and cucumber had begun to senesce, which is indicated by the lower reflectance in the NIR and SWIR regions and increase in reflectance in the VIS regions as chlorophyll is broken down. Absorbance in the VIS between 550 nm and 700 nm became less as both of these species began to senesce, reducing chlorophyll absorbance from 8 WAP to 10 WAP for cucumber and Palmer amaranth (Figure 2). Because Palmer amaranth and cucumber followed similar phenologic patterns throughout the season, they were often spectrally indistinguishable over many spectra (Supplementary Figures S7 and S8). At the time that cucumber and Palmer amaranth are beginning to senesce, peanut, soybean, and large crabgrass are beginning reproduction. Species that were phenologically different from one another were often more easily distinguishable, especially in the VIS, SWIR1, and SWIR2 regions. These include peanut and soybean (Supplementary Figures S4 and S5), sweetpotato and soybean (Supplementary Figures S5 and S6), and large crabgrass and cucumber (Supplementary Figures S9 and S10). Although phenology is not the only component of species differentiation, it plays a key role in seasonal changes in reflectance spectra.

Differences in plant phenology, such as the quick establishment of leaf canopies, the timing of plant emergence, the emergence of reproductive structures, or plant senescence, can be useful when using remote sensing to differentiate between species. Other studies have looked at spectral differences at distinct phenologic time points (Burks et al. 2002; Peña-Barragán et al. 2006), but the present study shows that spectra change with even minor changes in plant phenology. Because these phenologic changes between species are not aligned, changing phenology can be exploited for species separation but may not be consistent from year to year, based on environmental conditions. This approach does require knowledge of the biology and ecology associated with the species of interest, and plant phenology should be considered when using remote sensing for species differentiation.

Spectral Regions of Interest

Throughout the season the VIS region (350–700 nm) was important for species differentiation. Differentiation consistently occurred in pairwise comparisons of species in broad spectral regions around the blue (450 nm) and red (670 nm) (Supplementary Figures S1–S14) and are tied to plant chlorophyll content (Xue and Yang 2009). Some species in this study had a variable green color (namely, sweetpotato and nutsedge) around the green spectra (550 nm). In addition, NIR spectra (701–1300 nm), as previously mentioned, were important for early-season differentiation. SWIR1 and SWIR2, especially around peaks in these regions (1,550–1,690 nm and 2,025–2,200 nm for SWIR1 and SWIR2, respectively), in addition to VIS, can provide species differentiation and can change significantly throughout the season. The optimal time to take these measurements is between 2 and 5 WAP. Spectral differences between species in SWIR1 and SWIR2 were typically present by 5 WAP in both years. The SWIR1 and SWIR2 spectral regions have not been included in previous weed/crop differentiation research (Koger et al. 2004; López-Granados et al. 2008; Menges et al. 1985; Peña-Barragán et al. 2006), to our knowledge. The inclusion of these spectral regions in SWIR1 and SWIR2 for future sensor development could provide additional data that could be used for spectral differentiation and improve the accuracy of future site-specific management techniques.

### Canopy Versus Leaf-Level Spectra

As plant phenology changes throughout the season, biophysical changes such as cuticle thickness, anthocyanin accumulation, and canopy structure may affect reflectance spectra (Ahmadiani et al. 2016; Asner 1998; Dayan et al. 1996; Islam et al. 2002). Many of these biophysical changes can be detected on the leaf level, but not all of these changes translate to canopy-level spectra (Figures 1, 2, 5–8). Studies using only leaf-level spectra negate gross morphologic properties (e.g., leaf angle, plant canopy structure, the presence of inflorescence) but may provide additional information for species discrimination (Henry et al. 2004b; Vaiphasa et al. 2007). In the present study, with a 0.44-m^2 GIFOV, data collected from the canopy level comprised mixed spectra containing soil and foliage. The mixed spectra collected from the canopy accounts for the gross morphology and leaf arrangement of each species. Leaf-level measurements enable the determination of differences occurring within the measured leaf, removing effects of plant morphology. Results of this study demonstrate that certain species have unique spectra on the leaf level that were not always present in the canopy-level spectra.

Sweetpotato

Leaf-level spectra for sweetpotato exhibited unique reflectance when compared with other species in the study. Sweetpotato exhibited a notable absorption feature in upper leaves at 550 nm normally denoted by a peak in green reflectance (Wu et al. 2017) (Figures 7 and 8). New leaves of 'Covington' sweetpotato are often a burgundy color due to high levels of the anthocyanins cyanidin and peonidin (Ahmadiani et al. 2016; Islam et al. 2002), which have absorbance spectra at 540 nm and 546 nm, respectively. The presence of these anthocyanins in young sweetpotato leaves is likely the cause of the absorption feature around 550 nm
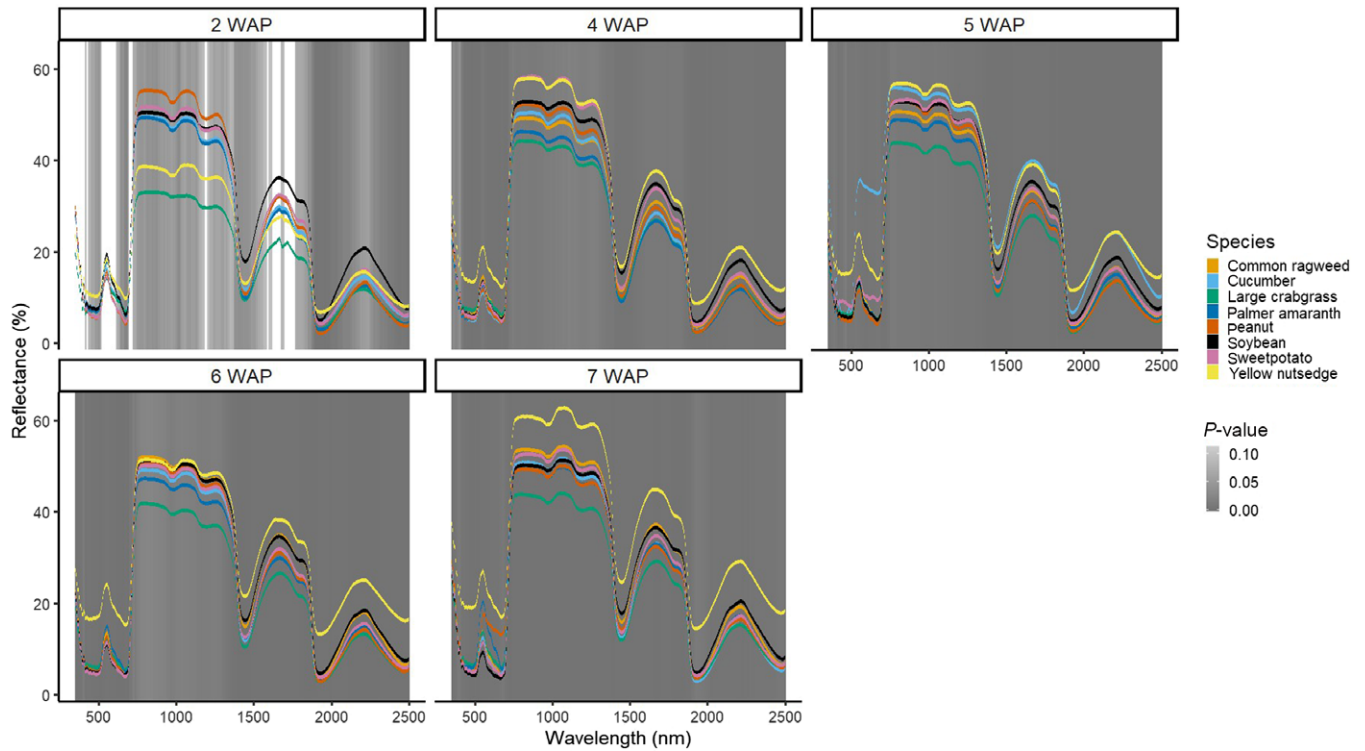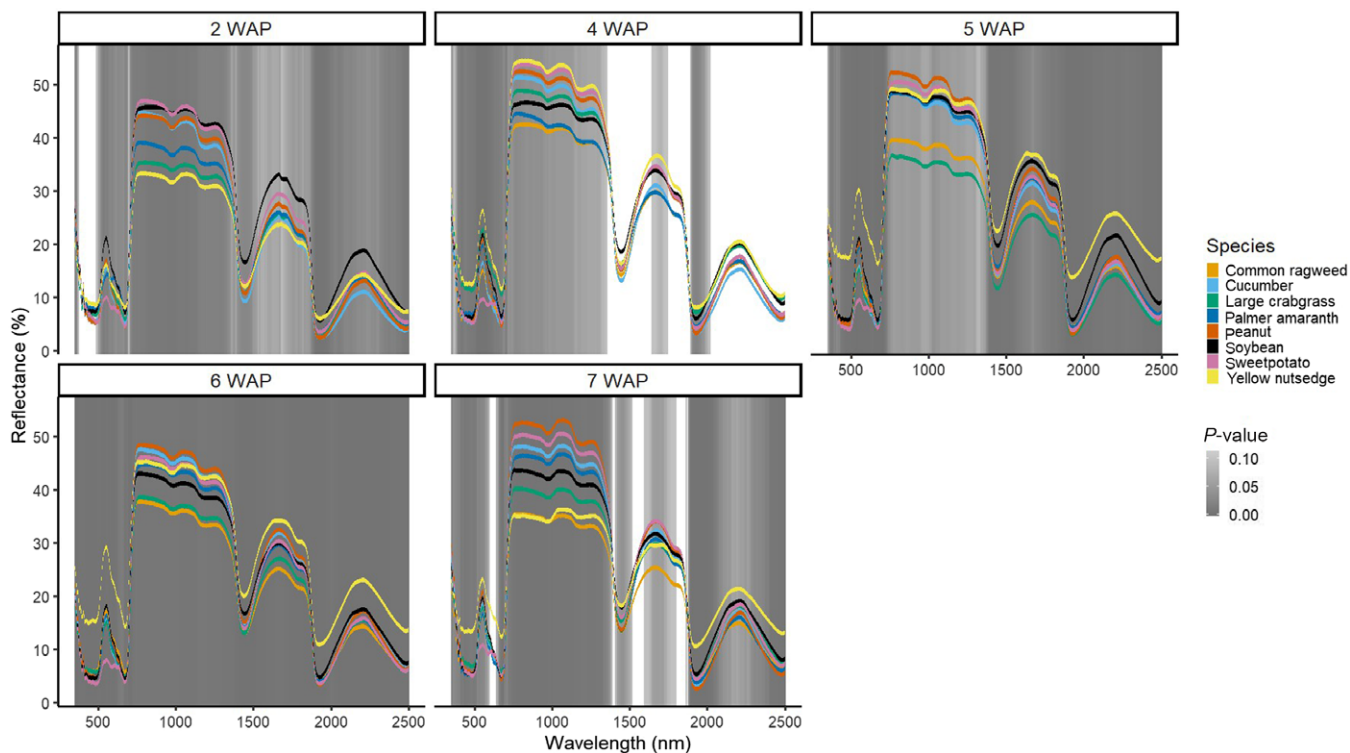
Figure 5. Lower leaf spectral reflectance curves for crop and weed species, Raleigh, NC, 2016. Species are indicated by color and reflectance spectra grouped by weeks after planting (WAP). Wavelengths at which species differences, as indicated by the Kruskal-Wallis at test P ≤ 0.1, are indicated as a continuous variable behind the spectral reflectance curves. Common ragweed spectra are not included at 2 WAP, because of slow weed emergence.
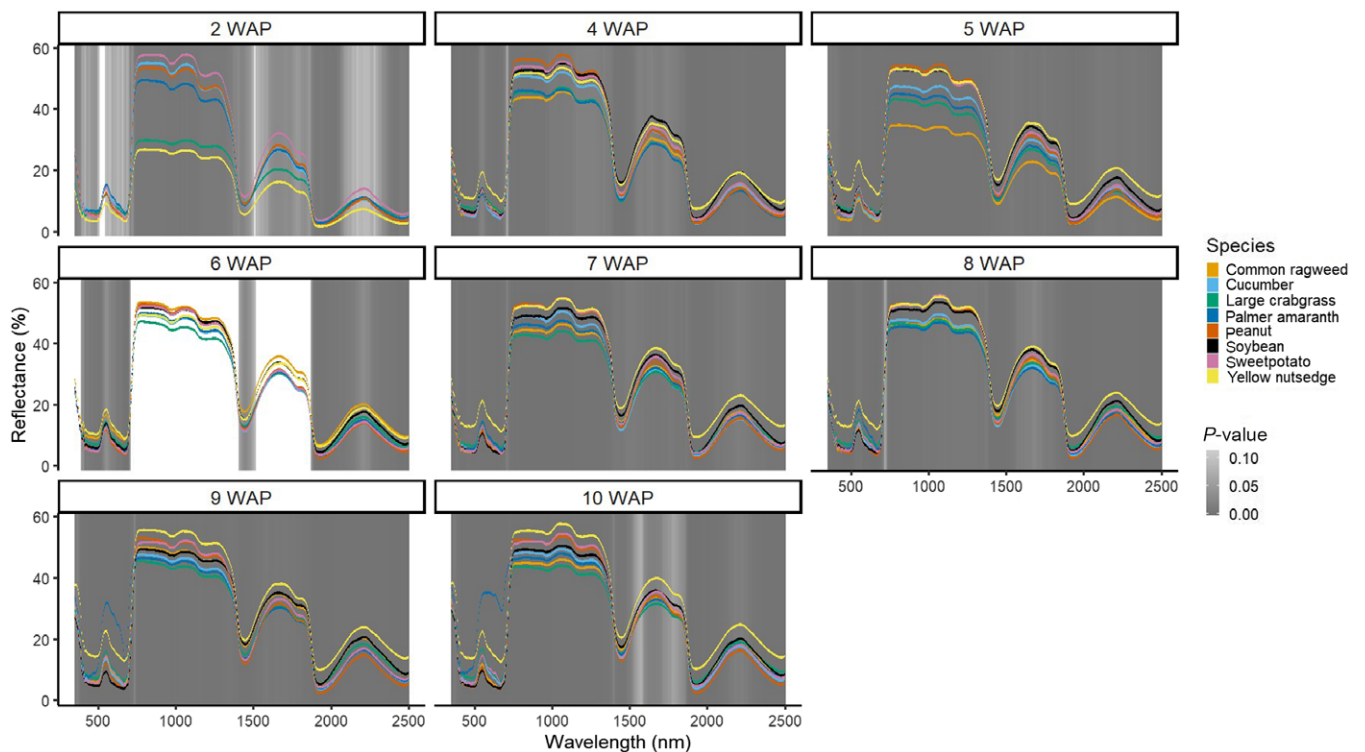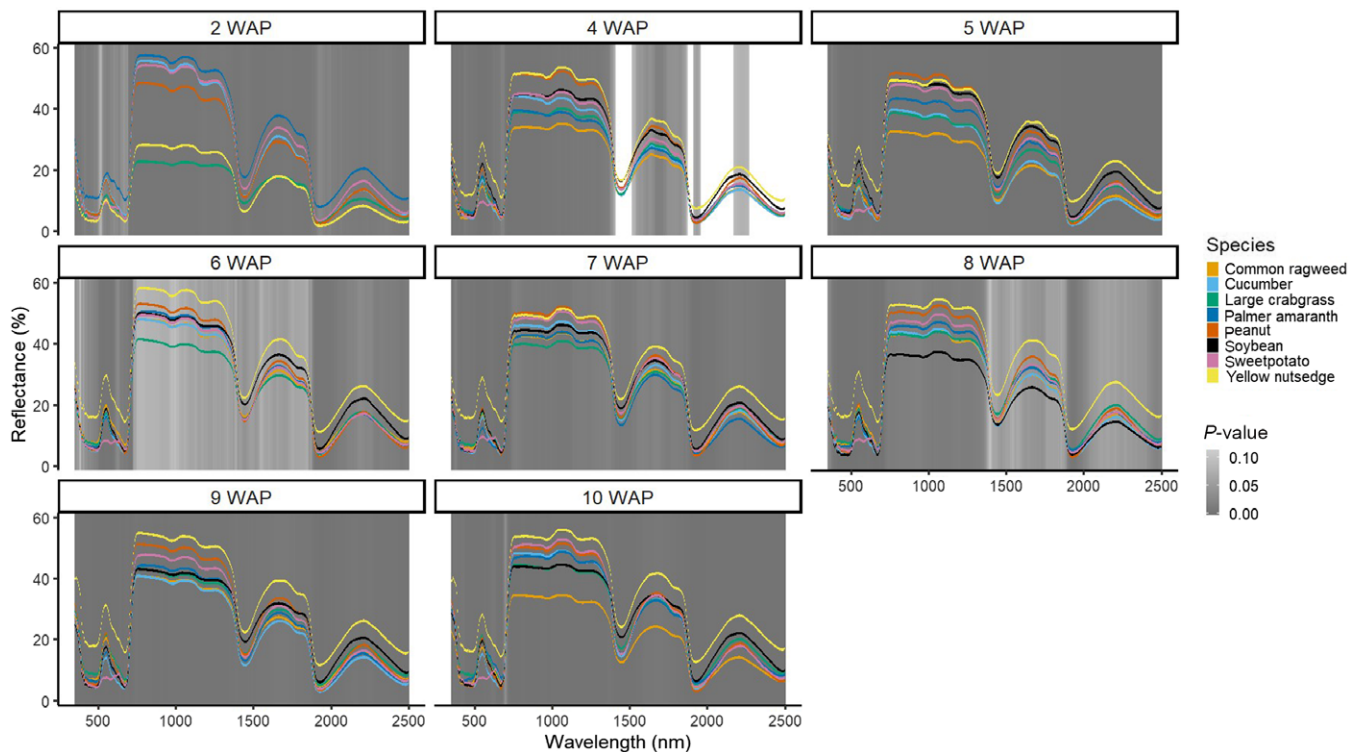

Figure 6. Lower leaf spectral reflectance curves for crop and weed species, Raleigh NC, 2017. Species are indicated by color and reflectance spectra grouped by weeks after planting (WAP). Wavelengths at which species differences, as indicated by the Kruskal-Wallis at test P ≤ 0.1, are indicated as a continuous variable behind the spectral reflectance curves. Common ragweed and soybean spectra are not included at 2 WAP, because of slow emergence.

Figure 7. Upper leaf spectral reflectance curves for crop and weed species, Raleigh NC, 2016. Species are indicated by color and reflectance spectra grouped by weeks after planting (WAP). Wavelengths at which species differences, as indicated by the Kruskal-Wallis at test P ≤ 0.1 are indicated as a continuous variable behind the spectral reflectance curves. Common ragweed spectra are not included at 2 WAP, because slow weed emergence.

Figure 8. Upper leaf spectral reflectance curves for crop and weed species, Raleigh, NC 2017. Species are indicated by color and reflectance spectra grouped by weeks after planting (WAP). Wavelengths at which species differences, as indicated by the Kruskal-Wallis at test P ≤ 0.1, are indicated as a continuous variable behind the spectral reflectance curves. Common ragweed and soybean spectra are not included at 2 WAP, because of slow emergence.
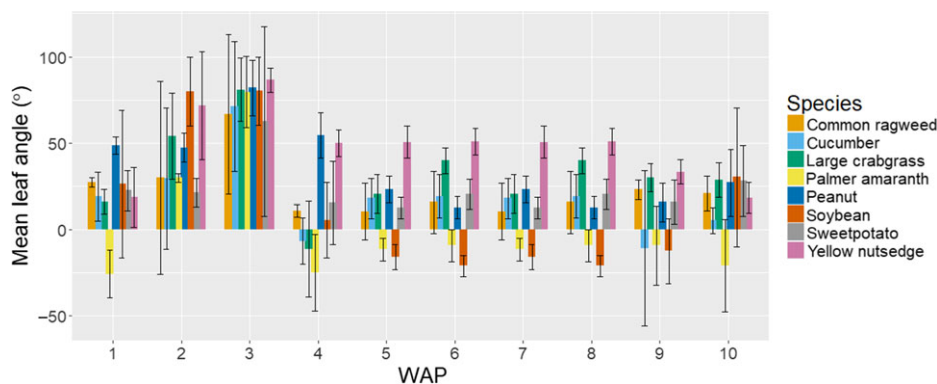
Figure 9. Weekly mean leaf angle for 10 wk after planting (WAP) for peanut, cucumber, soybean 'AG6535', sweetpotato 'Covington', and Palmer amaranth, common ragweed, yellow nutsedge, and large crabgrass, Raleigh, NC, 2017. A leaf angle of 0° is parallel with the soil surface. Positive angles are more erect leaf angles and negative angles are indicative of leaf angles below parallel.

(Asen et al. 1972; Rustioni et al. 2012). The concentration of anthocyanin in the young leaves act as photoprotectants, because anthocyanin concentration decreases with increased leaf shading (Islam et al. 2005). Leaves of 'Covington' sweetpotato transition to green as they mature. Despite the differences seen in the upper leaves of sweetpotato, the absorbance seen around 550 nm is not detected in the canopy-level reflectance (Figures 1 and 2). This could be due to the relatively low ratio of newly emerged burgundy leaves to mature green leaves. However, using spectra that are unique to specific crops or weeds could be exploited to provide additional parameters for species separation and improved site-specific management.

Yellow Nutsedge

In addition to unique spectra of sweetpotato, leaf-level spectra for yellow nutsedge tended to have higher overall reflectance across the VIS, SWIR1, and SWIR2 for upper and lower leaf measurements compared with other species in the study (Figures 5–8). Other researchers have noted that the leaf surface and visual appearance differ from that of other weed or crop species, including soybean (Dayan et al. 1996). Furthermore, yellow nutsedge in this study had high amounts of wax on the leaf surface. Low reflectance at 1 WAP for yellow nutsedge may be linked to a lack of development of these leaf traits. Brighter reflectance at measurements after 1 WAP may be related to this thick waxy cuticular layer in addition to a thick upper epidermis and uniformly distributed Kranz anatomy, which is common in C4 plants and distinguished by thick cell walls and chlorenchyma cells containing chloroplasts surrounding vascular bundles in the leaf (Wills 1987; Wills et al. 1980). The development of accessory and Kranz cells characterized by thicker cell walls (Wills et al. 1980) may increase light scattering, resulting in brighter reflectance in the VIS, SWIR1, and SWIR2 wavelengths. Differences observed in the SWIR1 and SWIR2 regions are often related to the development of lignin, cellulose, and starch in the plant (Youngentob et al. 2012). Previous studies (Cho et al. 2010; López-Granados 2011) were able to differentiate species without using spectra in the SWIR1 and SWIR2 bands. However, the results from the current research suggest that both crop and weed species respond differently in the SWIR areas of the reflectance spectra. As a result, these spectra could be used to differentiate between species.

Plant Morphology

Leaf angle for most species was not consistent across the season and leaves tended to be more erect from 1 to 3 WAP. As each

species matured, the leaf angle became more horizontal from 4 to 10 WAP, with some species having leaves angled down toward the soil surface (Figure 9). Other researchers have noted that reflectance spectra are influenced by leaf angle (Asner 1998), and changes in leaf angle with changing phenology may affect the differentiation of species over time (Andrew and Ustin 2009). In our study, plants that had horizontal foliage had similar spectral properties at the canopy level (Figures 1, 2, 9). Yellow nutsedge and large crabgrass, two species that have narrow erect leaves, were spectrally different at the upper and lower leaf level across measurement dates. Both species initially exhibited an erect growth habit. However, large crabgrass became more prostrate at 3 to 4 WAP, whereas yellow nutsedge maintained upright growth (Figure 9), resulting in changes in spectral reflectance at the canopy level. The influence of canopy structure on spectral reflectance has been observed in previous research (Asner 1998; Santos et al. 2012; Serbin et al. 2014; Xiao et al. 2014). Yellow nutsedge reflectance on the leaf level was high across the spectra measured (Figures 5–8) but had low reflectance in NIR and SWIR at the canopy level, indicating that canopy biophysical characteristics (Table 3; Figure 9) influenced overall reflectance (Figures 1 and 2). Also, the thickening of cuticular wax as leaves age (Yeats and Rose 2013) may contribute to changes in spectral reflectance (Lu 2013). This change in cuticular thickness could also explain differences in upper and lower leaves of these species, because lower leaves would have developed a thicker cuticular layer. It should also be noted that differences in gross morphology alone do not ensure that species are spectrally dissimilar. In this study, Palmer amaranth and cucumber were not differentiable across large regions of spectra (Supplementary Figures S7 and S8) despite very different plant morphology. Palmer amaranth is an upright weed (Horak and Loughlin 2006), whereas cucumber has a prostrate growth habit. The converse can also be true where species with similar morphology tend to have similar spectra. Cucumber and sweetpotato are both prostrate in growth habit but were spectrally similar in both years of the study (Supplementary Figures S13 and S14). Although others have noted that spectral differences may be tied to gross morphology, these spectral differences are likely tied to both plant morphology and phenology.

In this research, we have demonstrated that hyperspectral reflectance can be used to distinguish crop and weed species in situ. We have also further elucidated that small changes in phenology, in a little as a week, can affect plant spectra. In this study, species differentiation occurred across phenologic and

seasonal time points, which have been noted by others in limited agricultural (Peña-Barragán et al. 2006) and nonagricultural (Ouyang et al. 2013) settings. These changes in phenology can result in changes in leaf area, plant canopy structure, leaf color, or development of inflorescence or fruit. Plant phenology and biophysical characteristics have been linked to changes in hyperspectral reflectance spectra (Lausch et al. 2015; Ouyang et al. 2013; Schmidt and Skidmore 2003). Plants at very different phenology and/or with different morphology positively contribute to differences in species reflectance spectra but are not distinctly tied to one or the other. We have demonstrated that early-season spectra used for differentiation of species were often tied VIS and NIR spectra. However, as plants continued to mature, differences in species spectra remained differentiable in the VIS, but the SWIR1 and SWIR2 regions increased in importance. Furthermore, in this study, we have demonstrated that unique leaf-level spectra that could be used for species differentiation are not always present in canopy-level spectra. Differences in biophysical and phenologic characteristics between species could be used to further aid in separating weed and crop species from one another using spectroscopy. We also demonstrated that differentiation of species does have an important temporal component and that spectra, when collected at a closely timed temporal frequency, are more likely to catch phenologic stages at which species can be differentiated.

Differentiation between species was analyzed by wavelength and did not account for the spectral difference over the entirety of the measured spectra. Additional analysis methods considering the shape and magnitude of the whole spectral reflectance may provide additional insight into spectral variations between species, which may include using principal component analysis, Bhattacharyya distance, Jefferies-Matusita distance, or discriminant analysis. Because of the importance of the phenologic stage on differentiation in our study, future studies should consider phenology when investigating species differentiation. To continue to close the gap between data collection and management decisions, researchers should examine the impact of mixed species on differentiation. We believe that to make species discrimination for site-specific weed management a viable option in the future, integration of multiple disciplines (e.g., machine learning, remote sensing, and agronomy) is required.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wet.2020.92

References

Ahmadiani N, Robbins RJ, Collins TM, Giusti MM (2016) Molar absorptivity (ε) and spectral characteristics of cyanidin-based anthocyanins from red cabbage. Food Chem 197:900–906

Andrew ME, Ustin SL (2009) Habitat suitability modeling of an invasive plant with advanced remote sensing data. Divers Distrib 15:627–640

Andrew ME, Ustin SL (2008) The role of environmental context in mapping invasive plants with hyperspectral image data. Remote Sens Environ 112:4301–4317

Asen S, Stewart R N, Norris K H (1972). Co-pigmentation of anthocyanins in plant tissues and its effect on colour. Phytochemistry, 11, 1139–1144

Asner GP (1998) Biophysical and biochemical sources of variability in canopy reflectance. Remote Sens Environ 64:234–253

Bandyopadhyay KK, Pradhan S, Sahoo RN, Singh R, Gupta VK, Joshi DK, Sutradhar AK (2014) Characterization of water stress and prediction of yield of wheat using spectral indices under varied water and nitrogen management practices. Agric Water Manag 146:115–123

Basinger NT (2018) Interference and spectral changes of Palmer amaranth (*Amaranthus palmeri* S. Wats.) and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] density in sweetpotato and soybean and use of spectroscopy for discrimination of crop and weed species. Ph.D dissertation. Raleigh, NC: North Carolina State University. 989 p

Blackburn GA (2007) Hyperspectral remote sensing of plant pigments. J Exp Bot 58:855–867

Bolch EA, Santos MJ, Ade C, *et al.* (2020) Remote detection of invasive alien species. Pages 267–307 *in:* Cavender-Bars J, Gamon JA, Towsend PA (eds.). Remote Sensing of Plant Diversity. Berlin, Germany: SpringerOpen

Burks TF, Shearer SA, Green JD, Heath JR (2002) Influence of weed maturity levels on species classification using machine vision. Weed Sci 50:802–811

Burks TF, Shearer SA, Heath JR, Donohue KD (2005). Evaluation of neural-network classifiers for weed species discrimination. Biosyst Eng 91(3):293–304

Casanova D, Epema GF, Goudriaan J (1998) Monitoring rice reflectance at field level for estimating biomass and LAI. Field Crop Res 55:83–92

Cho MA, Debba P, Mathieu R, Naidoo L, Van Aardt J, Asner GP (2010) Improving discrimination of savannah tree species through a multiple-endmember spectral angle mapper approach: Canopy-level analysis. IEEE Trans Geosci Remote Sens 48:4133–4142

Cohen Y, Alchanatis V, Zusman Y, Dar Z, Bonfil DJ, Karnieli A, Zilberman A, Moulin A, Ostrovsky V, Levi A, Brikman R, Shenker M (2010) Leaf nitrogen estimation in potato based on spectral data and on simulated bands of the VENμS satellite. Precis Agric 11:520–537

Corder GW, Foreman DI (2009) *Nonparametric Statistics for Non-Statisticians.* Hoboken, NJ: John Wiley and Sons

Curran PJ (1989) Remote sensing of foliar chemistry. Remote Sens Environ 30:271–278

Dayan FE, Green HM, Weete JD, Hancock HG (1996) Postemergence activity of sulfentrazone: effects of surfactants and leaf surfaces. Weed Sci 44:797–803

Del Fiore A, Reverberi M, Ricelli A, Pinzari F, Serranti S, Fabbri AA, Bonifazi G, Fanelli C (2010) Early detection of toxigenic fungi on maize by hyperspectral imaging analysis. Int J Food Microbiol 144:64–71

Dian Bah M, Hafiane A, Canals R (2018) Deep learning with unsupervised data labeling for weed detection in line crops in UAV images. Remote Sens 10:1–23

Diker K, Bausch WC (2003) Potential use of nitrogen reflectance index to estimate plant parameters and yield of maize. Biosyst Eng 85:437–447

Ehleringer J (1983) Ecophysiology of *Amaranthus palmeri*, a Sonoran Desert summer annual. Oecologia 57:107–112

Everman WJ, Medlin CR, Dirks RD, Bauman TT, Biehl L (2008) The effect of postemergence herbicides on the spectral reflectance of corn. Weed Technol 22:514–522

Farooq A, Jia X, Hu J, Zhou J (2019) Multi-resolution weed classification via convolutional neural network and superpixel based local binary pattern using remote sensing images. Remote Sens 11:1692

Gamon JA, Serrano L, Surfus JS (1997) The photochemical reflectance index: an optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. Oecologia 112:492–501

Glenn NF, Mundt JT, Weber KT, Prather TS, Lass LW, Pettingill J (2005) Hyperspectral data processing for repeat detection of small infestations of leafy spurge. Remote Sens Environ 95:399–412

Goel PK, Prasher SO, Landry JA, Patel RM, Bonnell RB, Viau AA, Miller JR (2003) Potential of airborne hyperspectral remote sensing to detect nitrogen deficiency and weed infestation in corn. Comput Electron Agric 38:99–124

Gray CJ, Shaw DR, Bruce LM (2009) Utility of hyperspectral reflectance for differentiating soybean (*Glycine max*) and six weed species. Weed Technol 23:108–119

Hansen PM, Schjoerring JK (2003) Reflectance measurement of canopy biomass and nitrogen status in wheat crops using normalized difference vegetation indices and partial least squares regression. Remote Sens Environ 86:542–553

Hemming J, Rath T (2001) Computer-vision-based weed identification under field conditions using controlled lighting. J Agric Eng Res 78:233–243

Henry WB, Shaw DR, Reddy KR, Bruce LM, Tamhankar HD (2004a) Remote sensing to distinguish soybean from weeds after herbicide application. Weed Technol 18:594–604

Henry WB, Shaw DR, Reddy KR, Bruce LM, Tamhankar HD (2004b) Spectral reflectance curves to distinguish soybean from common cocklebur (*Xanthium strumarium*) and sicklepod (*Cassia obtusifolia*) grown with varying soil moisture. Weed Sci 52:788–796

Horak MJ, Loughin TM (2006) Growth analysis of four *Amaranthus* species. Weed Sci 48:347–355

Huang Y, Lee MA, Thomson SJ, Reddy KN (2016) Ground-based hyperspectral remote sensing for weed management in crop production. Int J Agric Biol Eng 9:98–109

Hung C, Xu Z, Sukkarieh S (2014) Feature learning based approach for weed classification using high resolution aerial images from a digital camera mounted on a UAV. Remote Sens 6:12037–12054

Hunt ER, Daughtry CST, Mirsky SB, Hively WD (2014) Remote sensing with simulated unmanned aircraft imagery for precision agriculture applications. IEEE J Sel Top Appl Earth Obs Remote Sens 7:4566–4571

Islam MS, Jalaluddin M, Garner JO, Yoshimoto M, Yamakawa O (2005) Artificial shading and temperature influence on anthocyanin compositions in sweetpotato leaves. HortScience 40:176–180

Islam MS, Yoshimoto M, Terahara N, Yamakawa O (2002) Anthocyanin compositions in sweetpotato (ipomoea batatas L.) leaves. Biosci Biotechnol Biochem 66:2483–2486

Jain N, Ray SS, Singh JP, Panigrahy S (2007) Use of hyperspectral data to assess the effects of different nitrogen applications on a potato crop. Precis Agric 8:225–239

Jensen JR (2007) *Remote Sensing of the Environment: An Earth Resource Persepective*. 2nd edn. Upper Saddle River, NJ: Prentice-Hall, Inc. 592 p

Jurado-Expósito M, López-Granados F, Atenciano S, García-Torres L, González-Andújar JL (2003) Discrimination of weed seedlings, wheat (*Triticum aestivum*) stubble and sunflower (*Helianthus annuus*) by near-infrared reflectance spectroscopy (NIRS). Crop Prot 22:1177–1180

Koger CH, Shaw DR, Reddy KN, Bruce LM (2004) Detection of pitted morning-glory (*Ipomoea lacunosa*) by hyperspectral remote sensing. I. Effects of tillage and cover crop residue. Weed Sci 52:222–229

Kokaly RF, Asner GP, Ollinger SV, Martin ME, Wessman CA (2009) Characterizing canopy biochemistry from imaging spectroscopy and its application to ecosystem studies. Remote Sens Environment 113: S78–S91

Lamb DW, Brown RB (2001) Remote-sensing and mapping of weeds in crops. J Agric Engng Res 78:117–125

Lass LW, Callihan RH (1997) The effect of phenological stage on detectability of yellow hawkweed (*Hieracium pratense*) and oxeye daisy (*Chrysanthemum leucanthemum*) with remote multispectral digital imagery. Weed Technol 11:248–256

Lausch A, Salbach C, Schmidt A, Doktor D, Merbach I, Pause M (2015) Deriving phenology of barley with imaging hyperspectral remote sensing. Ecol Model 295:123–135

López-Granados F (2011) Weed detection for site-specific weed management: mapping and real-time approaches. Weed Res 51:1–11

López-Granados F, Peña-Barragán JM, Jurado-Expósito M, Francisco-Fernández M, Cao R, Alonso-Betanzos A, Fontenla-Romero O (2008) Multispectral classification of grass weeds and wheat (*Triticum durum*) using linear and nonparametric functional discriminant analysis and neural networks. Weed Res 48:28–37

Lu S (2013) Effects of leaf surface wax on leaf spectrum and hyperspectral vegetation indices. Int Geosci Remote Sens Symp 453–456

Mahlein AK, Rumpf T, Welke P, Dehne HW, Plümer L, Steiner U, Oerke EC (2013) Development of spectral indices for detecting and identifying plant diseases. Remote Sens Environ 128:21–30

Medlin CR, Shaw DR, Gerard PD, LaMastus FE (2000) Using remote sensing to detect weed infestations in *Glycine max*. Weed Sci 48:393–398

Meier U (2001) *Growth Stages of Mono- and Dicotyledonous Plants*. 2nd edn. Bonn, Germany: Federal Biological Research Centre for Agriculture and Forestry

Menges RM, Nixon PR, Richardson AJ (1985) Light reflectance and remote sensing of weeds in agronomic and horticultural crops. Weed Sci 33:569–581

Ouyang ZT, Gao Y, Xie X, Guo HQ, Zhang TT, Zhao B (2013) Spectral discrimination of the invasive plant *Spartina alterniflora* at multiple phenological stages in a saltmarsh wetland. PLoS One 8:1–12

Peña-Barragán JM, López-Granados F, Jurado-Expósito M, García-Torres L (2006) Spectral discrimination of *Ridolfia segetum* and sunflower as affected by phenological stage. Weed Res 46:10–21

Penuelas J, Filella I, Biel C, Serrano L, Save R (1993) The reflectance at the 950-970 nm region as an indicator of plant water status. Int J Remote Sens 14:1887–1905

Ray SS, Das G, Singh JP, Panigrahy S (2006) Evaluation of hyperspectral indices for LAI estimation and discrimination of potato crop under different irrigation treatments. Int J Remote Sens 27:5373–5387

Rustioni L, Bedgood DR, Failla O, Prenzler PD, Robards K (2012) Copigmentation and anti-copigmentation in grape extracts studied by spectrophotometry and post-column-reaction HPLC. Food Chem 132:2194–2201

Santos MJ, Hestir EL, Khanna S, Ustin SL (2012) Image spectroscopy and stable isotopes elucidate functional dissimilarity between native and nonnative plant species in the aquatic environment. New Phytol 193:683–695

Schmidt KS, Skidmore AK (2003) Spectral discrimination of vegetation types in a coastal wetland. Remote Sens Environ 85:92–108

Serbin SP, Singh A, McNeil BE, Kingdon CC, Townsend PA (2014) Spectroscopic determination of leaf morphological and biochemical traits for northern temperate and boreal tree species. Ecol Appl 24:1651–1669

Thenkabail PS, Enclona EA, Ashton MS, Van Der Meer B, (2004) Accuracy assessments of hyperspectral waveband performance for vegetation analysis applications. Remote Sens Environ 80:213–224

Thorp KR, Tian LF (2004) A review on remote sensing of weeds in agriculture. Precis Agri 5:447–508

Ustin SL, Roberts DA, Gamon JA, Asner GP, Green RO (2004). Using imaging spectroscopy to study ecosystem processes and properties. Bioscience 54: 523–534

Vaiphasa C, Skidmore AK, de Boer WF, Vaiphasa T (2007) A hyperspectral band selector for plant species discrimination. ISPRS J Photogramm 62: 225–235

Webster TM (2014) Weed survey-southern states: broadleaf crops sub-section. Proceedings, Southern Weed Sci Soc 66:1–5

Webster TM (2010) Weed survey-southern states: vegetable, fruit and nut crops sub-section. Proceedings, Southern Weed Sci Soc 63:246–257

Wills GD (1987) Herbicide action on purple and yellow nutsedge (*Cyperus rotundus* and *C. esculentus*). Weed Technol 1:2–9

Wills GD, Hoagland RE, Paul RN (1980) Anatomy of yellow nutsedge (*Cyperus esculentus*). Weed Sci 28:432–437

Wu J, Chavana-Bryant C, Prohaska N, Serbin SP, Guan K, Albert LP, Yang X, van Leeuwen WJD, Garnello AJ, Martins G, Malhi Y, Gerard F, Oliviera RC, Saleska SR (2017) Convergence in relationships between leaf traits, spectra and age across diverse canopy environments and two contrasting tropical forests. New Phytol 214:1033–1048

Xiao Y, Zhao W, Zhou D, Gong H (2014) Sensitivity analysis of vegetation reflectance to biochemical and biophysical variables at leaf, canopy, and regional scales. IEEE Trans Geosci Remote Sens 52:4014–4024

Xue L, Yang L (2009) Deriving leaf chlorophyll content of green leafy vegetables from hyperspectral reflectance. ISPRS J Photogramm 64:97–106

Yeats TH, Rose JK (2013) The formation and function of plant cuticles. Plant Physiol 163:5–20.

Youngentob KN, Renzullo LJ, Held AA, Jia X, Lindenmayer DB, Foley WJ (2012) Using imaging spectroscopy to estimate integrated measures of foliage nutritional quality. Methods Ecol Evol 3:416–426.