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Detection of Nitrogen and Phosphorus Nutrient Status in Winter Wheat Using Spectral Radiance

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ABSTRACT

Nitrogen (N) and phosphorus (P) are major limiting nutrient elements for crop production and continued interest lies in improving their use efficiency. Spectral radiance measurements were evaluated to identify optimum wavelengths for dual detection of N and P status in winter wheat (*Triticum aestivum* L.). A factorial treatment arrangement of N and P (0, 56, 112, and 168 kg N ha⁻¹ and 0, 14.5, and 29 kg P ha⁻¹) was used to further study N and P uptake and associated spectral properties at Perkins and Tipton, Oklahoma. A wide range of spectral radiance measurements (345-1,145 nm) were obtained from each plot using a PSD1000 Ocean Optics fiber optic spectrometer. At each reading date, 78 bands and 44 combination indices were generated to test for correlation with forage biomass and N and P uptake. Additional spectral radiance readings were collected using an integrated sensor which has photodiode detectors

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and interference filters for red and NIR. For this study, simple numerator/denominator indices were useful in predicting biomass, and N uptake and P uptake. Numerator wavelengths that ranged between 705 and 735 nm and denominator wavelengths between 505 and 545 nm provided reliable prediction of forage biomass, and N and P uptake over locations and Feekes growth stages 4 through 6. Using the photodiode sensor, NDVI [(NIR-red)/(NIR+red)] and NR [(NIR/red)], were also good indices to predict biomass, and N and P uptake. However, no index was found to be good for detecting solely N and P concentration either using the spectrometer or photodiode sensor.

INTRODUCTION

Reports of surface and subsurface water contamination have led to the need for improved N and P fertilizer management. Due to the significance that N and P fertilizers have on crop production, application of these two fertilizer elements often exceed the recommended rate. A quick method to determine the status of N and P in wheat plant tissue could be valuable to improve N and P fertilizer management practices.

Use of spectral radiance as a tool to determine the nutrient element status in plants has several advantages compared to other non-destructive methods. Spectral radiance measurements can be obtained without attaching the meter to a specific leaf and many readings can be acquired in a short time thus reducing variability (Blackmer et al., 1994). In addition, sensors combined with other technologies, such as geographic information system (GIS) and global positioning system (GPS), should be able to make local specific fertilizer recommendations (Schepers, 1994). Therefore, undesired environmental effects associated with excess fertilization might be reduced.

Some indices have been proposed to predict biological growth (biomass) and nutrient element status in plant tissue. The normalized pigment chlorophyll index [NPCI = reflectance at 430 nm (R430) - reflectance at 680 nm (R680)/(R680 + R430)] was recently used to predict carotenoid/Chl A ratio (Penuelas et al., 1993). Similarly, the normalized difference vegetation index (NDVI = NIR - red/NIR + red) has provided good correlation with dry biomass (Mahey et al., 1991; Penuelas et al., 1993). Also, moisture in leaves can be predicted at 1,300 and 2,400 nm (Kleman and Fagerlund, 1987). However, less has been done concerning the use of spectral radiance measurements for detecting plant P status. Therefore, the objectives of this study were to (i) identify the optimum reflectance wavelength or indices for detecting dual N and P status in winter wheat and (ii) identify the ideal stage of growth for detecting N and P status.

MATERIALS AND METHODS

Two field experiments were conducted at Tipton (Tillman-Hollister clay loam, fine-loamy, mixed, thermic, Pachic Argiustoll) and Perkins (Teller sandy loam, fine-

TABLE 1. Initial surface (0-15 cm) soil test characteristics, Tipton and Perkins, OK, 1996.

Characteristics	Extractant	Unit	Tipton	Perkins
pH	1:1 soil:H ₂ O	-	7.8	5.9
Organic Carbon ^a	Dry Combustion	g/kg	7.158	5.336
Total Nitrogen ^a	Dry Combustion	g/kg	0.719	0.504
NH ₄ -N ^b	2 M KCl	mg/kg	3.0	3.0
NO ₃ -N ^b	2 M KCl	mg/kg	4.0	2.8
P ^c	Mehlich-3	mg/kg	43.9	8.9
K ^c	Mehlich-3	mg/kg	464.5	133.0

^aSchepers et al. (1989).

^bLachat Instruments (1989).

^cMehlich (1984).

loamy, mixed, thermic Udic Argiustoll), Oklahoma. Soil characteristics at each of these locations are reported in Table 1. A factorial arrangement of treatments for N and P rates was used at each location (0, 56, 112, and 168 kg N ha⁻¹ with 0, 14.5, and 29 kg P ha⁻¹). The experimental design was a randomized complete block with three replications with individual plots measuring 3.1 m x 9.1 m. Harvested area for forage sample was 0.5 m².

Spectrometer Readings

Spectral readings and forage yield were collected at Feekes growth stages 4, 5, 6, and 9 at Tipton and 5, 6, and 7 at Perkins (Large, 1954). A wide range of spectral radiance measurements (300 to 1,100 nm) were obtained from each plot using a PSD 1000 portable dual spectrometer manufactured by Ocean Optics, Inc. from two overlapping bandwidths, 300-850 nm and 650-1,100 nm. The PSD 1000 was connected to a portable computer through a PCMCIA slot using a PCM-DAS 16D/12 A/D converter manufactured by Computer Boards, Inc. The fiber optic spectrometer which has 200 µm diameter and no slit has spectral resolution as low as 5 nm. The bifurcated fiber was lifted with an hemispherical lucite™ lens which increased its angle of acceptance to 34°. The lens was held 1.5 m high and the area sensed was 0.8 m² per plot.

All spectral readings were partitioned into 10 nm bandwidths (78 spectral bands per reading). In addition, spectral indices, such as NDVI (Normalized Difference Vegetation Index) and other combinations of single indices, were generated. All

TABLE 2. Analysis of variance for wet biomass, dry biomass, moisture, P tissue concentration, P uptake, N tissue concentration, and N uptake in wheat forage at Feekes growth stages 4, 5, 6, and 9, Tipton, OK, 1997.

Source of variation	df	Wet biomass	Dry Biomass	Moisture	P tissue conc.	P tissue uptake	N tissue conc.	N uptake
Mean squares								
Rep	2	ns	ns	ns	*	ns	ns	ns
N rate	3	***	***	**	ns	***	ns	***
P rate	2	ns	ns	ns	ns	ns	ns	ns
NxP	6	ns	ns	ns	ns	ns	ns	ns
Error (a)	22	9267264	1630115	33	108191	13.0	8.9	666
GS	3	***	***	***	***	***	***	***
GSxN	9	***	***	***	ns	***	ns	***
GSxP	6	ns	ns	ns	ns	ns	ns	ns
GSxNxP	18	ns	ns	ns	ns	ns	ns	ns
Error (b)	71	8733477	1577047	17	96772	15.5	6.4	691
Means								
Growth Stage	N, kg/ha ⁻¹	kg/ha ⁻¹	kg/ha ⁻¹	%	mg/kg ⁻¹	kg/ha ⁻¹	g/kg ⁻¹	kg/ha ⁻¹
Feekes 4	0	4113	2112	49	2268	4.7	22.7	47.3
	56	4894	2425	50	2226	3.9	23.8	55.4
	112	4268	2165	49	2412	11.6	13.6	51.8
	168	5019	2503	49	2222	6.4	10.0	58.0
Feekes 5	0	3631	1745	51	2288	5.3	23.1	41.7
	56	4301	1967	53	2159	4.2	23.8	47.0
	112	4667	2145	53	2274	16.2	13.9	48.7
	168	5169	2478	51	2035	8.0	9.9	60.2
Feekes 6	0	6375	4701	24	2506	5.2	23.9	63.6
	56	10560	6987	31	2314	4.8	22.9	98.4
	112	18040	10517	40	2297	24.2	16.6	179.9
	168	18112	10526	41	2462	12.0	10.5	183.6
Feekes 9	0	9369	3071	67	2094	5.5	23.2	30.5
	56	12899	4050	68	1997	4.9	24.3	40.6
	112	21362	6601	69	1816	26.1	17.4	69.9
	168	23141	7083	69	2108	15.5	10.8	77.2
SED		1393	591	2	146	1.8	1.2	12.4
Growth Stage (over N and P rates)								
Feekes	4	4573	2301	49	2282	5.2	23.2	53.1
Feekes	5	4442	2084	52	2189	4.5	23.7	49.4
Feekes	6	13271	8183	34	2395	19.5	15.4	131.4
Feekes	9	16693	5201	68	2004	10.5	10.3	54.6
SED		696	296	1	73	0.9	0.6	6.2
P, kg/ha ⁻¹ (over N rates and growth stages)								
	0	9241	4240	50	2155	9.0	17.8	67.5
	14.5	10216	4582	52	2219	10.1	18.5	75.1
	29	9778	4505	51	2278	10.7	18.2	73.7
SED		603	256	1	63	0.8	0.5	5.3

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

GS=growth stage.

ns=not significant.

SED=standard error of the difference between two equally replicated means.

spectral radiance readings were standardized using a barium sulfate (BaSO_4) background reading. Statistical analysis was performed using SAS (SAS Institute, 1988).

Photodiode Sensor Readings

The collection of spectral radiance readings using the spectrometer was different from that obtained using the photodiode sensors. Photodiode spectral radiance readings were collected at growth stages Feekes 4, 5, 6, and 7 at Perkins and 5, 6, and 9 at Tipton from an area $0.19 \text{ m} (3 \text{ rows}) \times 0.91 \text{ m}$ long area.

Spectral radiance readings using photodiode sensors were obtained using an integrated sensor and signal processing system created by Stone et al. (1996). The integrated sensor has photodiode detectors and interference filters for red and NIR spectral bands with a 0.305 m by 0.075 m spatial resolution. This sensor allowed for red ($671 \pm 6 \text{ nm}$) and two kinds of NIR: long NIR ($1050 \pm 6 \text{ nm}$) and short NIR ($780 \pm 6 \text{ nm}$); therefore, NDVI [Normalized Difference Vegetation Index = $(\text{NIR-red}) / (\text{NIR} + \text{red})$] consisted of two indices (LNDVI and SNDVI). LNDVI refers to using long NIR in the NDVI formula and SNDVI refers to using short NDVI. The same is true for the simple ratio NR (NIR/red) where LNR refers to long NIR/red and SNR referred to short NIR/red.

Individual wavelengths and combination indices were evaluated to predict wet biomass, dry biomass, total N uptake, total P uptake, and total N and P concentration in winter wheat forage. All spectral radiance readings were standardized using a BaSO_4 background reading. Statistical analysis was performed using SAS (SAS Institute, 1988).

RESULTS AND DISCUSSION

At both sites, a significant increase in wheat forage yield due to applied N was observed at all stages of growth (Tables 2 and 3). Forage yield response to applied P was not significant at Tipton but was significant at Perkins. No N x P interaction was detected at either location. The lack of a P response at Tipton was due to high inorganic soil test P (Table 1). The main effect of growth stage was significant at both sites, a result of increased biomass with growth stage. Dry biomass decreased from Feekes growth stage 6 to 9 due to frost which caused tissue death and subsequent biomass loss.

Linear Regression

The three best indices (multi-wavelength combinations) to predict N rate, P rate, wet biomass, dry biomass, moisture, P concentration, P uptake, and N concentration and uptake were determined by growth stage (Tables 4 and 5). Consistent correlation over growth stages was found for numerator (705-735) and

denominator (505-545 nm) combinations when predicting N uptake at both locations. Other dependent variables did not result in consistent correlation with spectral indices over growth stages and locations. The use of denominator wavelengths that were not independently correlated with the dependent variable assisted in removing variability that was present when using the numerator wavelength alone.

Using data combined over all growth stages, the three best indices (single and multi-wavelength combinations) to predict N rate, P rate, wet biomass, dry biomass, moisture, P concentration, P uptake, and N concentration and uptake were determined (Table 6). No one index, either single or multi-wavelength combination was consistent over time in being correlated with N rate, P rate, wet and/or dry biomass, P concentration, P uptake and N concentration. However, over growth stages, consistent correlation from 10 nm bands between 625 and 695 nm was found with N uptake.

Spectrometer: Nitrogen Rate and Phosphorus Rate by Growth Stage

The indices that could be used to predict N rate varied (Tables 4 to 5). The highest correlation at Tipton was found at Feekes 5 with the index W715/545. Correlation tended to increase from Feekes 4 to Feekes 7. At Tipton, no index was significant to predict N rate at Feekes 4, but improved at later stages of growth. This indirectly suggests that percent foliage cover was critical in the establishment of indices for N status in winter wheat since the percent coverage was low when the first readings were taken at Tipton. As was expected, correlation of indices to predict P rate at Tipton was lower than at Perkins.

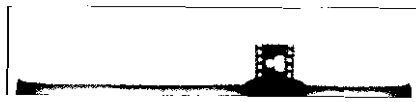
Spectrometer: Wet Biomass, Dry Biomass, and Moisture by Growth Stage

As has been demonstrated by others, NDVI has been commonly used to predict biomass in various studies (Mahey et al., 1991; Penuelas et al., 1993). However, we did not observe consistent correlation between NDVI and wet biomass (Tables 4 to 5). Dry biomass correlation with several indices increased with advancing stages of growth at both Tipton and Perkins. The index that was common to both locations in predicting dry biomass was W735/665.

Analysis by growth stage suggested that moisture in plants could not be adequately predicted until Feekes 5. The same observation was noted for biomass, indicating that a critical amount of coverage is needed in order to override background soil interference. However, results at Perkins were not consistent with observations at Tipton. At Perkins, moisture could be detected at earlier growth stages but not at later stages.

Spectrometer: Phosphorus Tissue Concentration, Phosphorus Uptake, Nitrogen Tissue Concentration, and Nitrogen Uptake by Growth Stage

Analysis by growth stage suggested that P concentration could be predicted at earlier growth stages using W705/505 at Perkins and NR at Tipton. However, at



Wxxx or Wxxx/xxx=index combinations using specified bandwidth; 0.18915=correlation coefficient; (0.2692)=probability > |R|; NPCIX=(W685-W435)/(W685+W435); NR=W805/W695; NW=W975-W905; NIRGI=W795/(1/W545); PRI=(W555-W535)/W555+W535; PFR=W725/655; NDVI=(W805-W695)/(W805+W695).

Variable	Model	Correlation Coefficient	Probability > R
Moisture	W735/655	0.2697	(0.1116)
	W735/655	0.26897	(0.1127)
	NDVI	0.2511	(0.1332)
P tissue conc.	NIR	0.42476	(0.0098)
	NW	-0.39688	(0.0165)
	W715/545	0.36387	(0.0291)
P uptake	W695/405	0.36901	(0.0268)
	W725/545	0.34808	(0.0375)
	NW	-0.34595	(0.0388)
N tissue conc.	NPCIX	0.31860	(0.0582)
	NR	0.31718	(0.0594)
	W735/655	-0.22597	(0.1851)
N uptake	W725/545	0.28006	(0.0981)
	W725/545	0.27875	(0.0997)
	W725/525	0.27609	(0.1031)

Dependent variables	1	2	3	1	2	3	1	2	3	
N rate	W735/655	0.25546	-0.25489	(0.1326)	(0.1336)	(0.1388)	W785/505	0.25161	0.92254	(0.0001)
	W735/545	0.25161	0.92254	(0.0001)	(0.0001)	(0.0001)	W715/545	0.15971	-0.16864	(0.0001)
	W735/545	0.15971	-0.16864	(0.0001)	(0.0001)	(0.0001)	W725/545	0.47612	0.47571	(0.0033)
P rate	W735/655	-0.21561	-0.21385	(0.2066)	(0.2104)	(0.2887)	W795/735	0.19322	-0.19322	(0.3251)
	W735/545	-0.21385	-0.21385	(0.2066)	(0.2104)	(0.2887)	NW	0.15971	-0.16864	(0.3251)
	W735/545	-0.21385	-0.21385	(0.2066)	(0.2104)	(0.2887)	W725/515	0.4803	0.49598	(0.0033)
Wet Biomass	NDVI	-0.21585	-0.20775	(0.2061)	(0.2241)	(0.2269)	W725/545	0.4803	0.49598	(0.0033)
	W725/505	-0.20775	-0.20775	(0.2061)	(0.2241)	(0.2269)	W735/515	-0.80198	-0.80426	(0.0001)
	W725/515	-0.20775	-0.20775	(0.2061)	(0.2241)	(0.2269)	W735/525	-0.79648	-0.79921	(0.0001)
Dry biomass	W695/405	0.34843	-0.26098	(0.0373)	(0.1242)	(0.2283)	W705/525	0.48096	-0.48096	(0.003)
	NDVI	0.34843	-0.26098	(0.0373)	(0.1242)	(0.2283)	W725/545	0.47612	0.47571	(0.0033)
	W725/545	0.47612	0.47571	(0.0033)	(0.0033)	(0.0033)	W735/515	-0.79648	-0.79921	(0.0001)

TABLE 4. Spectral radiance combinations and simple correlation from the three best models with N rate, P rate, biomass, moisture, P tissue concentration, P uptake, N tissue concentration, and N uptake by growth stage, Tipton, OK, 1997.

TABLE 7. Analysis of variance for Red, LNIR, SNIR, LNDVI, SNDVI, LNR, and SNR in wheat forage at Feckes growth stages 5, 6, and 9, Tipton, OK, 1997.

Source of variation	df	Red	LNIR	SNIR	LNDVI	SNDVI	LNR	SNR
Rep	2	ns	ns	ns	ns	ns	ns	ns
N rate	3	***	ns	ns	***	***	***	***
P rate	2	ns	ns	ns	ns	ns	ns	ns
NXP	6	ns	ns	ns	ns	ns	ns	ns
Error (a)	22	22732	17778	286612	0.03	0.01	0.45	49.55
GS	2	***	ns	ns	***	***	***	***
GSxN	6	***	ns	ns	***	***	***	***
GSxP	4	ns	ns	ns	ns	ns	ns	ns
GSxNXP	12	ns	ns	ns	ns	ns	ns	ns
Error (b)	48	19003	13168	216721	0.02	0.00	0.36	61.75
GS	0	601	386	1609	-0.24	0.45	0.65	2.74
Feckes 5	0	637	446	1919	-0.19	0.49	0.72	3.11
	56	543	378	1647	-0.21	0.49	0.71	3.09
	168	547	387	1737	-0.18	0.52	0.74	3.35
Feckes 6	0	449	382	1956	-0.15	0.62	0.84	4.42
	56	270	331	1896	0.07	0.76	1.46	9.01
	112	291	291	1868	0.29	0.88	2.78	25.47
	168	377	377	2154	0.48	0.90	3.96	25.02

Feckes 9	0	56	112	168	SEd	Feckes	5	6	9	SEd	P rate	0	503	434	2281	2220	421	433	31	126
	971	509	2746	-0.31	0.48	0.55	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	56	512	2827	-0.18	0.58	0.72	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	168	589	3434	0.20	0.80	1.65	9.64	8.10	9.64	3.70	3.07	3.07	582	399	1728	1968	345	544	27	109
	SEd	54	219	0.06	0.03	0.28	3.70	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	0	509	2746	-0.31	0.48	0.55	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	56	512	2827	-0.18	0.58	0.72	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	168	589	3434	0.20	0.80	1.65	9.64	8.10	9.64	3.70	3.07	3.07	582	399	1728	1968	345	544	27	109
	SEd	54	219	0.06	0.03	0.28	3.70	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	0	509	2746	-0.31	0.48	0.55	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	56	512	2827	-0.18	0.58	0.72	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	168	589	3434	0.20	0.80	1.65	9.64	8.10	9.64	3.70	3.07	3.07	582	399	1728	1968	345	544	27	109
	SEd	54	219	0.06	0.03	0.28	3.70	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	0	509	2746	-0.31	0.48	0.55	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	56	512	2827	-0.18	0.58	0.72	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	168	589	3434	0.20	0.80	1.65	9.64	8.10	9.64	3.70	3.07	3.07	582	399	1728	1968	345	544	27	109
	SEd	54	219	0.06	0.03	0.28	3.70	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	0	509	2746	-0.31	0.48	0.55	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	56	512	2827	-0.18	0.58	0.72	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	168	589	3434	0.20	0.80	1.65	9.64	8.10	9.64	3.70	3.07	3.07	582	399	1728	1968	345	544	27	109
	SEd	54	219	0.06	0.03	0.28	3.70	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	0	509	2746	-0.31	0.48	0.55	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	56	512	2827	-0.18	0.58	0.72	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	168	589	3434	0.20	0.80	1.65	9.64	8.10	9.64	3.70	3.07	3.07	582	399	1728	1968	345	544	27	109
	SEd	54	219	0.06	0.03	0.28	3.70	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	0	509	2746	-0.31	0.48	0.55	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	56	512	2827	-0.18	0.58	0.72	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	168	589	3434	0.20	0.80	1.65	9.64	8.10	9.64	3.70	3.07	3.07	582	399	1728	1968	345	544	27	109
	SEd	54	219	0.06	0.03	0.28	3.70	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	0	509	2746	-0.31	0.48	0.55	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	56	512	2827	-0.18	0.58	0.72	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	168	589	3434	0.20	0.80	1.65	9.64	8.10	9.64	3.70	3.07	3.07	582	399	1728	1968	345	544	27	109
	SEd	54	219	0.06	0.03	0.28	3.70	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	0	509	2746	-0.31	0.48	0.55	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	56	512	2827	-0.18	0.58	0.72	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	168	589	3434	0.20	0.80	1.65	9.64	8.10	9.64	3.70	3.07	3.07	582	399	1728	1968	345	544	27	109
	SEd	54	219	0.06	0.03	0.28	3.70	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	0	509	2746	-0.31	0.48	0.55	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	56	512	2827	-0.18	0.58	0.72	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	168	589	3434	0.20	0.80	1.65	9.64	8.10	9.64	3.70	3.07	3.07	582	399	1728	1968	345	544	27	109
	SEd	54	219	0.06	0.03	0.28	3.70	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	0	509	2746	-0.31	0.48	0.55	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	56	512	2827	-0.18	0.58	0.72	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	168	589	3434	0.20	0.80	1.65	9.64	8.10	9.64	3.70	3.07	3.07	582	399	1728	1968	345	544	27	109
	SEd	54	219	0.06	0.03	0.28	3.70	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	0	509	2746	-0.31	0.48	0.55	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	56	512	2827	-0.18	0.58	0.72	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	168	589	3434	0.20	0.80	1.65	9.64	8.10	9.64	3.70	3.07	3.07	582	399	1728	1968	345	544	27	109
	SEd	54	219	0.06	0.03	0.28	3.70	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	0	509	2746	-0.31	0.48	0.55	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	56	512	2827	-0.18	0.58	0.72	3.01	4.07	8.10	9.64	3.70	3.07	582	399	1728	1968	345	544	27	109
	168	589	3434	0.20	0.80	1.65	9.64	8.10	9.64	3.70	3.07	3.07	582	399	1728	1968	345	544	27</	

***Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
 GS=growth stage.
 ns=not significant.
 SED=standard error of the difference between two equally replicated means.

Rep	N rate	P rate	NXP	Error (a)	GS	GSxN	GSxP	GSxNXP	Error (b)
2	ns	3	2	6	22	3	9	6	18
3	***	***	***	ns	28682	***	***	ns	8563
4	***	***	***	ns	12915	***	***	ns	3417
5	ns	***	***	*	448126	***	***	ns	66813
6	ns	***	***	ns	0.07	***	*	ns	0.01
7	ns	***	***	ns	0.02	***	*	ns	0.00
8	ns	***	***	ns	0.45	***	*	ns	0.09
9	ns	***	***	ns	14.14	***	*	ns	2.85
10	ns	***	***	ns	5.11	0.99	1.18	6.07	3.04
11	5.90	1.20	1.88	6.49	2.481	0.64	1.26	6.49	3.04
12	9.95	1.20	1.88	6.07	2.481	0.64	1.18	6.07	3.04
13	11.53	2.15	2.15	6.49	2.570	0.71	1.26	6.49	3.04
14	5.56	1.00	1.00	3.14	1.532	0.51	0.39	3.14	3.04
15	6.87	1.16	1.16	4.48	1.880	0.61	0.62	4.48	3.04
16	12.47	2.17	2.17	4.88	2.202	0.72	0.95	4.88	3.04
17	14.06	2.52	2.52	6.61	2.202	0.72	0.95	6.61	3.04
18	0.80	0.15	0.15	7.87	2.515	0.74	1.18	7.87	3.04

Mean squares

Source of variation	df	Red	LNR	SNR	LNDVI	SNDVI	LNR	SNR
Rep	2	*	ns	ns	ns	ns	ns	ns
N rate	3	***	***	***	***	***	***	***
P rate	2	**	ns	ns	**	**	**	**
NXP	6	ns	ns	*	ns	ns	ns	ns
Error (a)	22	28682	12915	448126	0.07	0.02	0.45	14.14
GS	3	***	***	***	***	***	***	***
GSxN	9	**	**	**	*	ns	***	***
GSxP	6	ns	ns	ns	ns	ns	*	*
GSxNXP	18	ns	ns	ns	**	ns	ns	ns
Error (b)	71	8563	3417	66813	0.01	0.00	0.09	2.85

TABLE 8. Analysis of variance for Red, LNR, SNR, LNDVI, SNDVI, LNR, and SNR in wheat forage at Feekes growth stages 4, 5, 6, and 9, Perkins, OK, 1997.

later growth stages, none of these indices could predict P concentration (Tables 4 to 5). It may be that P deficiencies were not detected at later stages of growth due to increased root proliferation in profiles where subsoil P was greater.

At Tipton, P uptake could be predicted using W695/405 at Feekes growth stage 4 but this did not hold true at later growth stages. At Perkins, P uptake could be predicted using W715/505. Similarly, this index was not reliable at later growth stages. The same indices for predicting P uptake at Perkins were not always good at Tipton and *visa versa*. Similar to N uptake, correlation between P uptake and several of the spectral indices evaluated tended to increase with advancing stages of growth.

At Tipton, N concentration could not be reliably predicted at Feekes growth stage 4 but could be predicted at later growth stages. The same trend was observed at Perkins. However, indices were not consistently the same at Tipton and Perkins (Tables 4 to 5). It was interesting to note that W735/545 at Perkins and PFR at Tipton appeared at Feekes growth stages 5 and 6. In addition, W695/405 was also observed at both locations between Feekes growth stages 5 and 6.

At Tipton, N uptake could not be reliably predicted at Feekes growth stage 4 but could be predicted at later growth stages. This same trend was observed at Perkins. Although correlation increased with time, indices were not consistently the same at Tipton and Perkins (Tables 4 to 5). This indirectly suggests that beginning at Feekes 5, top-dress applications (based on growth) could be applied, given that a yield response was achieved using a highly correlated index with N uptake. In addition, W725/525 was highly correlated with N uptake at growth stages Feekes 4 and 9 at Tipton and at Feekes 5 at Perkins. Because of the similarities in numerator and denominator wavelength, the use of broad bands may need to be further evaluated.

Spectrometer: Nitrogen Rate and Phosphorus Rate over Growth Stages

Similar to the "by growth stage results," W725/525 (± 20 nm for both the numerator and denominator) provided good prediction of N rate over growth stages. Using all data over growth stages, no index could be used to reliably predict P rate at Tipton, however, at Perkins P rate could be predicted using W705/535, W705/545, and W705/525 (Table 6). The inconsistency of this observation by growth stage limits the utility.

Spectrometer: Wet Biomass, Dry Biomass, and Moisture over Growth Stages

There were several indices that reliably predicted wet biomass including W705/505, W715/515, and W715/505 at Tipton and 735/665, NPCIX, and W735/655 at Perkins. Dry biomass could be predicted by W705/515 and NPCIX at Tipton and Perkins, respectively (Table 6). Using all data combined over growth stages, the highest correlation coefficient for predicting moisture was with W795/735 at Tipton and W705/505 for Perkins.

Spectrometer: Phosphorus Tissue Concentration, Phosphorus Uptake, Nitrogen Tissue Concentration, and Nitrogen Uptake over Growth Stages

Using all data combined over growth stages, the highest correlation for predicting P concentration and P uptake was achieved with W785/505 and W705/545 at Tipton and NR and PFR at Perkins (Table 6).

The highest correlation for predicting N concentration was W705/505 at Tipton and W735/535 at Perkins. Nitrogen uptake was highly correlated with W795/735 and PFR at Tipton and Perkins, respectively.

Photodiode Sensors versus Agronomic Variables

Analysis of variance and associated means from spectral properties determined at various growth stages are reported in Tables 7 and 8 for Tipton and Perkins, respectively. Wet biomass was highly correlated with NDVI (LNDVI and SNDVI) at all growth stages at both locations. Correlation coefficients from linear regression with wet biomass ranged from 0.56 to 0.93 for SNDVI, 0.64 to 0.95 for LNDVI, 0.44 to 0.97 for LNR, and 0.58 to 0.97 for SNR (data not reported). Similarly, these same indices were highly correlated with dry biomass. Moisture tended to be less correlated with these same indices.

Combined over growth stage, tissue P concentration could not be reliably predicted with an index. However, P concentration could be predicted at Perkins at Feekes growth stages 4 and 5 using NDVI and NR. In addition, red was also a reliable predictor at earlier growth stages compared to later. Phosphorus uptake could be predicted using NDVI and NR at various stages of growth.

At Perkins and Tipton, N concentration was highly correlated with several of the indices evaluated, however, consistency over time was not observed. NDVI and NR were consistently correlated with N uptake over growth stages and locations. This suggests that using N uptake is better than just using tissue N concentration. Similar to results from spectrometer readings, combinations of wavelengths within indices provided superior correlation with dependent agronomic variables compared to single wavelengths.

Grain Yield versus Spectral Indices

Analysis of variance and associated means for grain yield, grain N, grain N uptake, grain P, and grain P uptake at Tipton and Perkins are reported in Table 9. It was found that only grain N and grain N uptake were affected by N rate. Grain N levels increasing with increasing N applied at both sites, even though no yield response was detected. Grain yield levels were low at both sites (late frost at Tipton and Perkins).

Correlation of grain yield, grain N, grain N uptake, grain P, and grain P uptake with spectral indices is reported in Table 10. It was interesting to note that grain N uptake was highly correlated with SNDVI, SNR, and LNR at Feekes stage 5 at

TABLE 9. Analysis of variance for grain, grain N uptake, grain N, grain P uptake, grain P, grain P uptake, Tipton and Perkins, OK, 1997.

Source of variation	df	Grain yield	Grain N uptake	Grain N uptake	Grain P uptake	Grain P uptake
Rep	2	ns	ns	ns	ns	ns
N rate	3	ns	***	ns	ns	ns
P rate	2	ns	ns	ns	ns	ns
NXP	6	ns	ns	ns	ns	ns
N lin	1	ns	***	*	ns	ns
N quad	1	ns	ns	ns	ns	ns
P lin	1	ns	ns	ns	ns	ns
P quad	1	ns	ns	ns	ns	ns
Error	22	9339	1.6	8.4	872862	0.3
CV, %		18	4	19	20	23
-----Mean squares-----						
N, kg ha ⁻¹		482	27.9	13.4	4453	2.1
0		519	27.8	14.4	4803	2.5
56		530	28.8	15.3	4895	2.6
112		548	30.5	16.8	4087	2.2
168		548	30.5	16.8	4087	2.2
SED		45	0.6	1.37	440	0.3
P, kg ha ⁻¹		495	28.9	14.4	4682	2.3
0		495	28.9	14.4	4682	2.3
14.5		530	28.9	15.3	4441	2.3
29		534	28.4	15.2	4556	2.3
SED		39	0.5	1.2	381	0.2

Source of variation	df	Grain yield	Grain N uptake	Grain N uptake	Grain P uptake	Grain P uptake
Rep	2	ns	ns	ns	ns	ns
N rate	3	ns	***	ns	ns	ns
P rate	2	ns	ns	ns	ns	ns
NXP	6	ns	ns	ns	ns	ns
N lin	1	ns	***	ns	ns	ns
N quad	1	ns	ns	ns	ns	ns
P lin	1	ns	ns	ns	ns	ns
P quad	1	ns	ns	ns	*	ns
Error	22	24378	4.5	26.4	241857	0.9
CV, %		20	7	23	12	30
-----Mean squares-----						
N, kg ha ⁻¹		828	26.6	22.1	4136	3.4
0		828	26.6	22.1	4136	3.4
56		721	27.5	19.7	4069	3.0
112		789	29.3	23.2	3901	3.1
168		786	31.2	24.4	4094	3.3
SED		74	1.0	2.4	232	0.4
P rate, kg ha ⁻¹		772	29.5	22.6	3808	3.0
0		772	29.5	22.6	3808	3.0
14.5		795	28.5	22.7	4311	3.4
29		777	27.9	21.7	4032	3.1
SED		64	0.9	2.1	201	0.4

*, **, ***Significant at the 0.05, 0.01, and 0.001 probability levels, respectively. ns=not significant. SED=standard error of the difference between two equally replicated means.



ns=not significant.
*, **, ***, ****Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
GS=growth stage.
0.18915=correlation coefficient.

Dependent variables	GS	Red	LNR	SNR	LNDVI	SNDVI	LNR	SNR
Grain yield	0.28 ^{ns}	0.50*	0.16 ^{ns}	0.48*	0.35 ^{ns}	0.26 ^{ns}	0.24 ^{ns}	0.25 ^{ns}
FecKes 4	-0.23 ^{ns}	0.12 ^{ns}	-0.26 ^{ns}	0.50*	0.15 ^{ns}	0.24 ^{ns}	0.21 ^{ns}	0.23 ^{ns}
FecKes 5	0.12 ^{ns}	0.16 ^{ns}	-0.26 ^{ns}	-0.38*	0.19 ^{ns}	0.30 ^{ns}	0.18 ^{ns}	0.36*
FecKes 6	0.16 ^{ns}	0.08 ^{ns}	-0.23 ^{ns}	0.19 ^{ns}	0.23 ^{ns}	0.18 ^{ns}	0.17 ^{ns}	0.36*
FecKes 9	0.08 ^{ns}	0.23 ^{ns}	-0.23 ^{ns}	-0.39*	0.19 ^{ns}	0.30 ^{ns}	0.18 ^{ns}	0.36*
Grain N	0.06 ^{ns}	0.04 ^{ns}	-0.16 ^{ns}	-0.07 ^{ns}	0.12 ^{ns}	0.05 ^{ns}	0.14 ^{ns}	0.39*
FecKes 7	0.31 ^{ns}	0.04 ^{ns}	-0.16 ^{ns}	-0.07 ^{ns}	0.12 ^{ns}	0.05 ^{ns}	0.14 ^{ns}	0.39*
FecKes 6	0.13 ^{ns}	0.13 ^{ns}	-0.16 ^{ns}	-0.07 ^{ns}	0.12 ^{ns}	0.05 ^{ns}	0.14 ^{ns}	0.39*
FecKes 5	0.13 ^{ns}	0.13 ^{ns}	-0.16 ^{ns}	-0.07 ^{ns}	0.12 ^{ns}	0.05 ^{ns}	0.14 ^{ns}	0.39*
FecKes 4	0.06 ^{ns}	0.04 ^{ns}	-0.16 ^{ns}	-0.07 ^{ns}	0.12 ^{ns}	0.05 ^{ns}	0.14 ^{ns}	0.39*
Grain N uptake	-0.50 ^{ns}	-0.46**	-0.09 ^{ns}	-0.39*	0.29 ^{ns}	0.42*	0.47**	0.46**
FecKes 7	-0.29 ^{ns}	-0.50 ^{ns}	-0.09 ^{ns}	-0.39*	0.29 ^{ns}	0.42*	0.47**	0.46**
FecKes 6	-0.19 ^{ns}	0.36*	0.36*	0.32*	0.35*	0.38*	0.34*	0.33*
FecKes 5	-0.28 ^{ns}	0.31 ^{ns}	0.36*	0.19 ^{ns}	0.04 ^{ns}	-0.03 ^{ns}	0.07 ^{ns}	0.07 ^{ns}
FecKes 4	-0.09 ^{ns}	0.55****	0.37 ^{ns}	0.53****	0.62****	0.59****	0.62****	0.61****
Grain P uptake	-0.26 ^{ns}	-0.23 ^{ns}	-0.29 ^{ns}	-0.33*	0.11 ^{ns}	0.19 ^{ns}	0.18 ^{ns}	0.23 ^{ns}
FecKes 7	0.21 ^{ns}	0.51 ^{ns}	0.49 ^{ns}	0.02 ^{ns}	0.22 ^{ns}	0.25 ^{ns}	0.26 ^{ns}	0.28 ^{ns}
FecKes 6	0.26 ^{ns}	0.13 ^{ns}	0.15 ^{ns}	0.36 ^{ns}	0.20 ^{ns}	0.15 ^{ns}	0.23 ^{ns}	0.20 ^{ns}
FecKes 5	0.21 ^{ns}	0.51 ^{ns}	0.49 ^{ns}	0.02 ^{ns}	0.22 ^{ns}	0.25 ^{ns}	0.26 ^{ns}	0.28 ^{ns}
FecKes 4	0.23 ^{ns}	0.36 ^{ns}	0.38 ^{ns}	0.19 ^{ns}	0.04 ^{ns}	-0.03 ^{ns}	0.07 ^{ns}	0.07 ^{ns}
Grain P uptake	-0.10 ^{ns}	-0.11 ^{ns}	0.01 ^{ns}	0.37*	0.41*	0.40*	0.42*	0.44**
FecKes 7	-0.23 ^{ns}	0.08 ^{ns}	0.12 ^{ns}	0.37*	0.41*	0.40*	0.42*	0.44**
FecKes 6	0.08 ^{ns}	0.38*	0.37*	0.35*	0.37*	0.34*	0.41*	0.44**
FecKes 5	0.23 ^{ns}	0.41*	0.41*	0.35*	0.37*	0.34*	0.41*	0.44**
FecKes 4	0.23 ^{ns}	0.41*	0.41*	0.35*	0.37*	0.34*	0.41*	0.44**

Dependent variables	GS	Red	LNR	SNR	LNDVI	SNDVI	LNR	SNR
Grain yield	-0.26 ^{ns}	-0.09 ^{ns}	0.02 ^{ns}	0.12 ^{ns}	0.27 ^{ns}	0.13 ^{ns}	0.27 ^{ns}	0.27 ^{ns}
FecKes 5	-0.26 ^{ns}	-0.09 ^{ns}	0.02 ^{ns}	0.12 ^{ns}	0.27 ^{ns}	0.13 ^{ns}	0.27 ^{ns}	0.27 ^{ns}
FecKes 6	-0.38*	-0.44*	-0.39*	-0.39*	0.29 ^{ns}	0.42*	0.47**	0.46**
FecKes 9	-0.38*	-0.44*	-0.39*	-0.39*	0.29 ^{ns}	0.42*	0.47**	0.46**
Grain N uptake	-0.56****	0.37*	0.37*	0.53****	0.62****	0.59****	0.62****	0.61****
FecKes 9	-0.56****	0.37*	0.37*	0.53****	0.62****	0.59****	0.62****	0.61****
FecKes 6	-0.53 ^{ns}	-0.19 ^{ns}	-0.11 ^{ns}	-0.11 ^{ns}	0.41*	0.51**	0.54****	0.39*
FecKes 5	-0.53 ^{ns}	-0.19 ^{ns}	-0.11 ^{ns}	-0.11 ^{ns}	0.41*	0.51**	0.54****	0.39*
FecKes 4	-0.16 ^{ns}	-0.16 ^{ns}	-0.07 ^{ns}	-0.07 ^{ns}	0.12 ^{ns}	0.05 ^{ns}	0.14 ^{ns}	0.39*
Grain N	-0.16 ^{ns}	-0.16 ^{ns}	-0.07 ^{ns}	-0.07 ^{ns}	0.12 ^{ns}	0.05 ^{ns}	0.14 ^{ns}	0.39*
FecKes 9	-0.23 ^{ns}	0.08 ^{ns}	0.19 ^{ns}	0.19 ^{ns}	0.23 ^{ns}	0.18 ^{ns}	0.17 ^{ns}	0.36*
FecKes 6	-0.23 ^{ns}	0.08 ^{ns}	0.19 ^{ns}	0.19 ^{ns}	0.23 ^{ns}	0.18 ^{ns}	0.17 ^{ns}	0.36*
FecKes 5	-0.26 ^{ns}	-0.09 ^{ns}	0.02 ^{ns}	0.12 ^{ns}	0.27 ^{ns}	0.13 ^{ns}	0.27 ^{ns}	0.27 ^{ns}
Grain P uptake	-0.25 ^{ns}	0.00 ^{ns}	-0.29 ^{ns}	-0.33*	0.11 ^{ns}	0.19 ^{ns}	0.18 ^{ns}	0.23 ^{ns}
FecKes 9	-0.25 ^{ns}	0.00 ^{ns}	-0.29 ^{ns}	-0.33*	0.11 ^{ns}	0.19 ^{ns}	0.18 ^{ns}	0.23 ^{ns}
FecKes 6	-0.23 ^{ns}	0.08 ^{ns}	0.19 ^{ns}	0.19 ^{ns}	0.23 ^{ns}	0.18 ^{ns}	0.17 ^{ns}	0.36*
FecKes 5	-0.23 ^{ns}	0.08 ^{ns}	0.19 ^{ns}	0.19 ^{ns}	0.23 ^{ns}	0.18 ^{ns}	0.17 ^{ns}	0.36*
FecKes 4	-0.08 ^{ns}	-0.02 ^{ns}	-0.07 ^{ns}	-0.02 ^{ns}	0.09 ^{ns}	0.03 ^{ns}	0.13 ^{ns}	0.07 ^{ns}

TABLE 10. Spectral radiance measurements, combination indices, and simple correlation with grain yield, grain N, grain N uptake, grain P, grain P uptake over growth stages, Tipson and Perkins, OK, 1997.

Perkins and 6 at Tipton. However, no index could reliably predict grain P and grain P uptake.

A positive relationship between forage N uptake and a spectral properties at early stages of growth does not guarantee that it will be correlated with grain yield. It is suggested that an experiment be conducted where fertilizer rates are determined based on spectral properties at different growth stages to document time of top-dressing when using indirect measures.

CONCLUSIONS

Spectral indices with numerator wavelengths that ranged between 705 and 735 nm and denominator wavelengths between 505 and 545 nm provided reliable prediction of wheat forage biomass, N and P uptake over locations and Feekes growth stages 4 to 6. It was found that NDVI and NR were good indices for the prediction of biomass, and N and P uptake, however, no index could reliably predict N and P concentration either using the spectrometer or sensor. Grain N uptake could be predicted using SNDVI, SNR, and LNR from spectral readings collected at Feekes stage 5. This finding was encouraging since there are many biological and environmental variables that can impact grain yield between Feekes stage 5 and physiological maturity.

This work demonstrates the difficulty in identifying constant indices that can be used over time for predicting chemical and biological parameters using spectrometer readings. One particular index is sometimes good at a given reading but not for others. Some of the problems encountered with using spectral properties to predict biological properties of plants include light intensity, weed pressure, clouds, and the sensitivity of the sensor in capturing the images of the plant canopy. In addition, part of the problem might be the resolution at which sensed data is collected.

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