Comparison of Traditional and Novel Non-destructive Sampling Techniques for Site-specific Assessment of Botanical Composition in Grass-Legume Pastures

Lisa L. Baxter and Charles P. West

TEXAS TECH UNIVERSITY

Introduction

- To better manage forage productivity, it is important to accurately and objectively monitor legume content in grass-legume pastures.
  - Currently rely on subjective visual assessments or labor-intensive botanical hand separations.
- Recent research has led to developments in photometric techniques. In addition, Biewer et al. (2009) distinguished legumes from binary grass mixtures using hyperspectral canopy reflectance measurements.
- Objective: to determine the predictive accuracy of photographic (ImageJ; Microsoft® PowerPoint, PPT) and in-field, reflectance-based procedures in assessing botanical composition in grass-legume pastures.

Materials and Methods

Forage mixtures:

1. Old World bluestem-alfalfa-yellow sweetclover [OWB, *Bothriochloa bladhii* (Retz) S.T. Blake; *Medicago sativa* L.; *Melilotus officinalis* L.].
2. Tall wheatgrass-alfalfa [TW, *Thinopyrum ponticum* (Podp.)]

Fieldwork:

1. Nine canopy reflectance measurements made in a 3 x 3 grid.
   - Black Comet CXR-SR hyperspectral spectrometer fitted with SMA-905 fiber optic cable (StellarNet, Inc., Tampa, FL) and Gershun tube kit (Ocean Optics, Inc., Dunedin, FL).
   - Full field of view 14^0 (0.09-m^2 sensing footprint ; Fig. 1).
   - Data saved on laptop as .TRM files.
   - Data collected on clear sky days within 2 hr ± local solar noon.
2. Sample area defined with 1-m^2 PVC quadrat.
3. Legume content visual estimated to nearest 10% of total biomass.
4. Images recorded using a Canon PowerShot D30 digital camera (Canon, Inc., Tokyo, Japan) mounted on a PVC monopod at 1.5 m high.
5. Sample area clipped to 10-cm stubble height, botanically separated, dried, and weighed.

Digital procedures:

1. Reflectance data converted from .TRM into .XLSX files.
2. Digital images were cropped to remove quadrat boundary before uploaded into PPT, overlain with grid, and each point defined as grass, legume, or soil (Rayburn, 2014; Fig. 2a-b).
3. Images processed in batches (by sampling date) using unique macros developed for ImageJ (version Fiji; imagej.nih.gov; Fig. 3a-c).

Statistical procedures:

- All data were analyzed in SAS 9.4 (SAS Inc., Cary, NC).
- Dependent variables (predicted legume content from visual, PPT, and ImageJ procedures) were linearly regressed on botanical hand separations (traditional procedure) using PROC AUTOREG.
- Reflectance data were smoothed in PROC EXPAND before first-order derivative was calculated. PROC PLS with a six-fold split-sample cross validation was used to determine optimum model. Final linear models fit in PROC AUTOREG.

Figure 1. Sensor mounting design for spectrometer creates 0.09-m^2 sensing footprint.

Figure 2. (a) Cropped sample image and (b) sample image under analysis in PowerPoint.

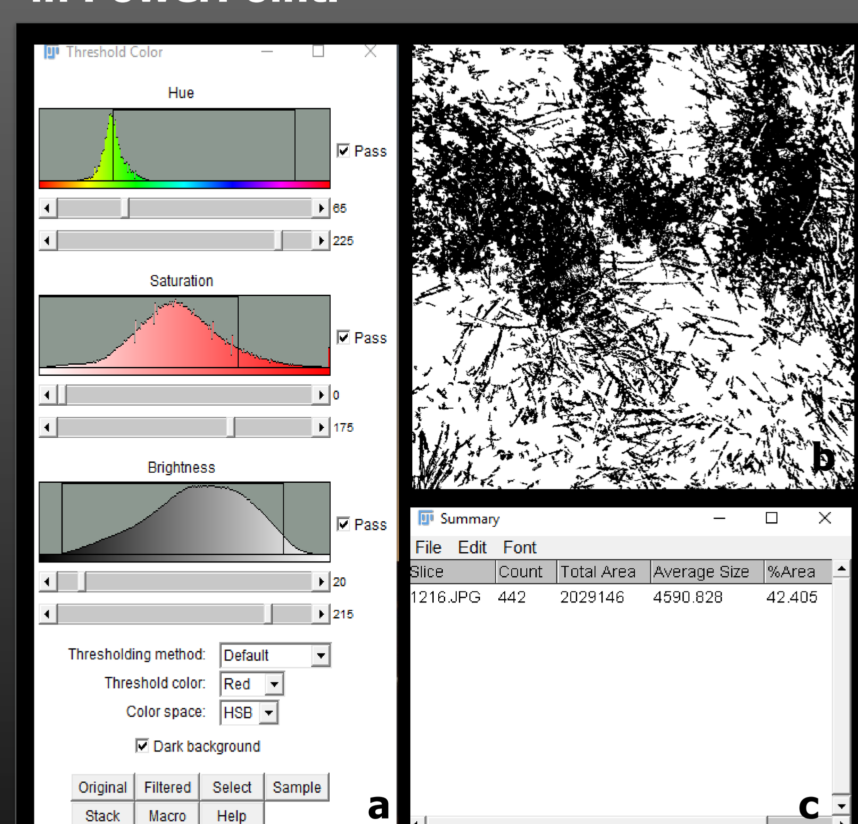

Figure 3. Hue, saturation, and brightness thresholds set in ImageJ (a) create binary images (b) from which pixel data are quantitatively extracted and summarized (c).

Results

Table 1. Results from regressing five sampling techniques on botanical separation for prediction of legume content in old world bluestem-legume pasture.

Sampling technique	n	R^2
Visual	481	0.80
PowerPoint	480	0.82
ImageJ	462	0.65
Canopy Reflectance (Smoothed Data)	266	0.58
Canopy Reflectance (1st Order Derivative)	266	0.55

Table 2. Results from regressing five sampling techniques on botanical separation for prediction of legume content in tall wheatgrass-alfalfa pasture.

Sampling technique	n	R^2
Visual	498	0.31
PowerPoint	498	0.45
ImageJ	498	0.17
Canopy Reflectance (Smoothed Data)	330	0.30
Canopy Reflectance (1st Order Derivative)	330	0.34

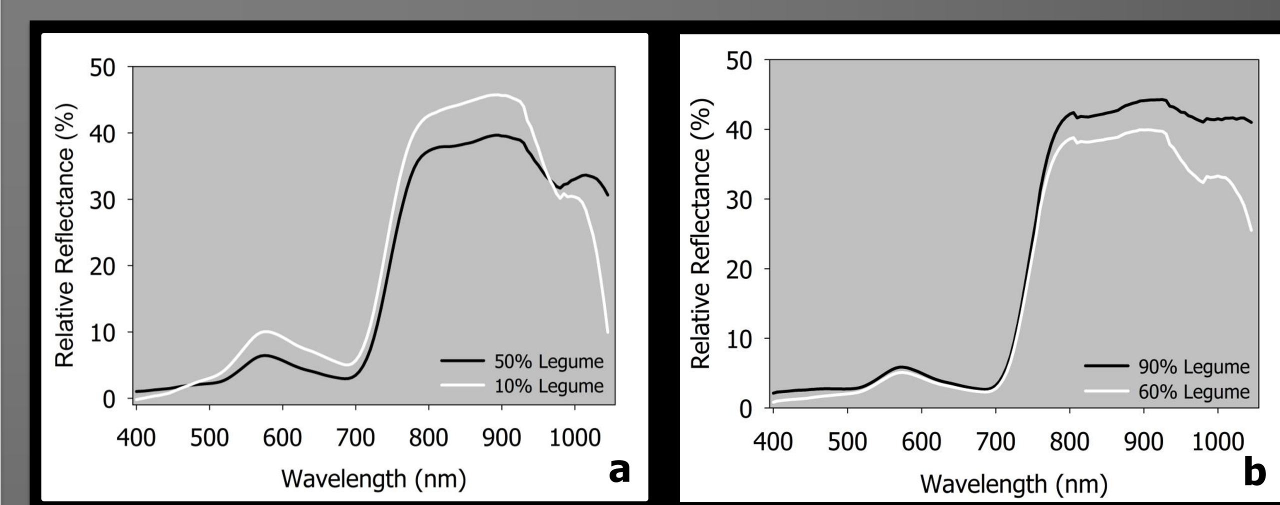

Figure 4. Example spectral curves using smoothed data. (a) Legumes are distinguished from old world bluestem at 695, 760, 800, and 845 nm. (b) Alfalfa was discriminated from tall wheatgrass at 675, 690, 695, and 750 nm.

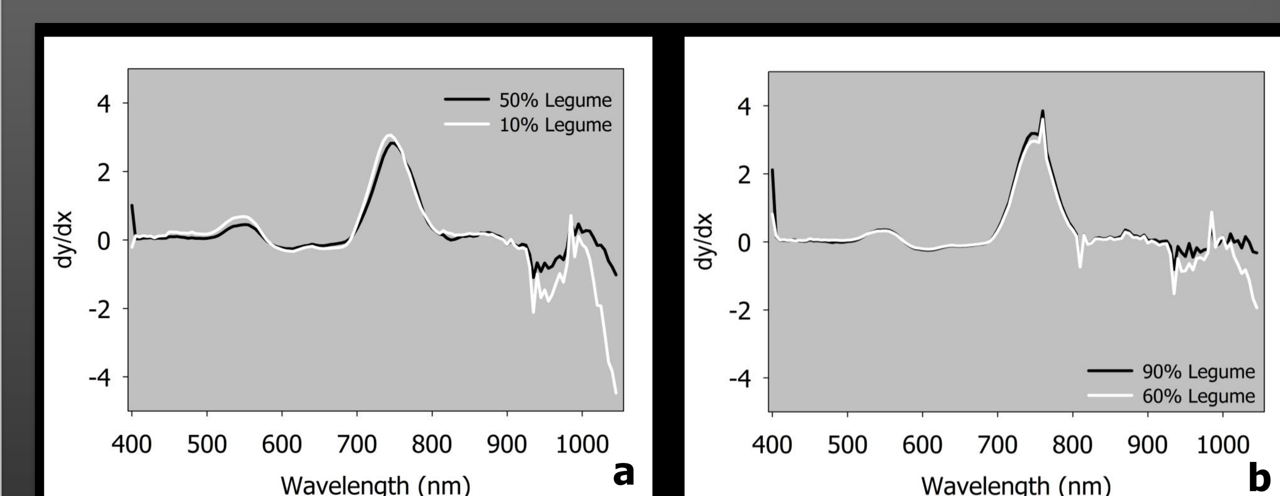

Figure 5. Example spectral curves using first derivative of smoothed data. (a) Rate changes at 620 and 680 nm corresponded to higher legume content in old world bluestem-legume. (b) Rate changes at 685, 690, 695, and 750 nm were used to determine alfalfa content in tall wheatgrass-alfalfa pasture.

Discussion

- Visual and PPT analyses were most successful in OWB-legume pasture (Table 1).
  - While visual analysis required less time, the PPT analysis provided *quantitative* estimates.
- None of the techniques were sufficient in determining legume content in TW-alfalfa (Table 2).
  - These forages are very similar shades of green which makes it difficult to estimate legume content visually, on-screen, or using spectral thresholds.
- A potential downfall of digital analyses is they rely on 2-D images and cannot account for sward height or canopy structure.
- Reflectance-based analyses (smoothed data) indicated legumes were best distinguished from grasses in the far-red and red-edge regions (Fig. 4a-b).
  - Reflectance generally corresponds to chlorophyll absorption and N concentration.
- Analysis on the first-order derivative of the reflectance curves detect differences in rate of change along the curve.
  - Increasing legume content in OWB-legume pasture lowered the rate near absorption of chlorophyll b yet increased near absorption of chlorophyll a (Fig. 5a).
  - Increasing alfalfa in TW-alfalfa pasture lowered the rate in the far-red region. This is consistent with higher N concentration (Fig. 5b). Changes in red-edge region correspond to rates of chlorophyll absorption (Fig. 5b).

Conclusions

The accuracy of non-destructive sampling techniques is promising for mixed pastures when the grass and legume components are distinct hues of green.

Although the need for more research is recognized, the ultimate goal is to apply these findings to automated scanners that offer producers rapid measurements of legume cover in mixed pastures.

Selected References

Biewer, S., S. Erasmi, T. Fricke, and M. Wachendorf. 2009. Precision Agric. 10:128-144.

Rayburn, E.B. 2014. Forage and Grazinglands. doi: 10.2134/FG-2011-0143-MG

FUNDING FOR THIS RESEARCH IS PROVIDED BY SARE (LS14-261) AND THE HELEN DEVITT JONES GRADUATE RESEARCH FELLOWSHIP.