

## Measuring Legume Content in Pastures Using Digital Photographs

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#### Abstract

Quantifying botanical composition is important for evaluating the effects of management on legume content and of legume content on pasture yield and quality. The standard for measuring botanical composition is hand separation of clipped samples. An alternative is taking point counts of botanical components on photographs of the pasture. The latter was tested on a rotationally stocked pasture, with photos taken at 24 random sample areas, areas clipped at ground level, and samples hand separated into grass, legume, and forb fractions. Photos were evaluated with a grid in Microsoft PowerPoint. Point counts were calibrated to hand-separated values using linear regression. Grass and legume point-count components were not significantly different from hand-separated values (P = 0.05) but underestimated the forb fraction. Calibration regressions had  $R^2$  values ranging from 0.45 to 0.98. The precision of this technique is dependent on the number of photos per pasture, the number of points counted per photo, and the number of paired samples taken for calibration. In cool-season grass-clover pastures, 12 or more photos per pasture and 100 or more points per photo are a good balance between photo number and points per photo. For calibration, 12 or more paired samples should be used. Photo point counts appear to be a practical method of measuring grass, legume, and forb components in rotationally grazed pastures.

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**Abbreviations:** Cl, confidence interval; SD, standard deviation.

#### INTRODUCTION

QUANTIFYING the botanical composition in pastures is important for measuring the effects of management on legume content and the effect of legume content on forage yield and quality. Quantifying legume content in pastures is also important since it can have a significant impact on animal performance. In Virginia, orchardgrass–clover pastures produced the same steer gain per acre as orchardgrass alone fertilized with 200 lbs. N/acre/year (Blaser et al., 1969), but at a lower stocking rate and higher gain per head. In West Virginia, 30% legumes in pastures increased backgrounding calf average Published in Forage and Grazinglands DOI 10.2134/FG-2011-0143-MG © 2014 American Society of Agronomy and Crop Science Society of America 5585 Guilford Rd., Madison, WI 53711

All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Permission for printing and for reprinting the material contained herein has been obtained by the publisher. daily gain by 0.50 lbs./day over that of straight grass pastures (Rayburn et al., 2006)

Clipping and hand separation of samples is the standard method for evaluating botanical composition in pastures; however, this method is very time consuming, so it is seldom used. Other methods include dry-weight rank, DAFOR (dominant, abundant, frequent, occasional, rare ranking) scale, point-intercept, and line-intercept methods (Mannetje and Haydock, 1963; Abaye et al., 1995, 1997; Interagency Technical Team, 1996; Rayburn et al., 2007). An alternative method is using a digital camera to take vertical photos of the pasture and conducting point counts of identifiable botanical components on the photographs. These photo point counts give a direct measure of the sward surface cover. A set of paired photo-point-count and clipped, hand-separated samples allows calibration of the photo-point-count data to dry-matter fraction of the botanical components.

The objective of this research is to determine if pasture botanical composition can be accurately measured using point counts of botanical components on digital photographs.

### **METHODS**

A rotationally stocked pasture of orchardgrass (*Dactylis glomerata*, L.), white clover (*Trifolium repens* L.), and red clover (*T. pratense* L.) was sampled with 24 paired photos and clipped samples. Sampling was done prior to turning cattle into the pasture after the pasture had grown to an 8- to 10-inch height. Photo points were selected

at random by walking an established sampling transect across the pasture. When reaching a photo point, a 12by 12-inch square quadrat with 8-inch legs was placed to mark the photo area (Fig. 1). A legged quadrat was used to prevent distortion of the pasture canopy. A digital photograph was taken of the area within the quadrat. Photos were taken with a handheld camera so that the area marked by the quadrat filled most of the photo. Photos were taken on a slightly overcast day to prevent direct sunlight reflecting off the leaves and causing glare. When conducting this work on sunny days, a white photographer's parasol can be used to prevent direct sunlight from causing glare.

After photographing the sample area, the legged quadrat was removed and a legless quadrat of the same size was placed on the ground. All tillers and leaves arising within the quadrat were moved to the inside, and those arising outside the quadrat were moved to the outside. The forage within the quadrat was then clipped at ground level. The clipped samples were bagged, taken to the lab, and frozen. The clipped samples were later thawed and hand separated into grass, legume, forb, and dead fractions. Botanical fractions from each sample area were dried at 140°F to determine component dry-matter yield.

Point counts of botanical components in photos were conducted with Microsoft PowerPoint presentation software. To make the point counts on a photo:

- 1. A point count grid of V's was constructed (Fig. 1).
- 2. A selected photo was imported into the software.
- 3. The point count grid was laid over the photo and moved to randomize the grid points (Fig. 1).



Figure 1. Digital photograph imported into Microsoft PowerPoint with a point-count grid overlying the photo.

- 4. At the end of each "V" within the quadrat, the points touching grass, legume, forb, bare ground, or dark shadows were categorized and counted.
- 5. The point count grid was then moved and randomized for additional counts until the desired number of points had been counted.
- 6. The fraction of points that contact the identified botanical component, bare ground, and dark shadow areas was then calculated. Where dark shadows prevented identifying what was at that point, the count was adjusted by dropping those points from the count.

Mean pasture botanical surface cover as measured by photo point count was compared with botanical composition measured by hand separation with a *t*-test (P = 0.05) (Hintze, 1998). Botanical cover measured by photo point count were also regressed (Hintze, 1998) against composition measured by hand separation to determine if they differed across the range of botanical components measured and to develop a calibration equation for adjusting photo-point-count cover to equivalent hand-separated composition for photos not clipped and hand separated.

#### RESULTS

The botanical composition of grass and legume fractions measured by photo point counts was not significantly different (P = 0.05) from that measured by hand separation when expressed as a fraction of live material (Table 1). However, the photo measurement of forb surface cover underestimated forb dry matter in the stand measured by hand separation. When measured as a fraction of total plant material, including dead material at the bottom of the canopy, all hand-separated botanical values were lower, and the point count of forb cover was not significantly different from hand-separated botanical composition. In this vegetative, rotationally stocked pasture, dead material was at the bottom of the canopy, where it could not be seen on the photos. Since livestock usually are not forced to eat dead material at the bottom of the canopy, measuring botanical composition on a live-material basis is preferable.

Using regression analysis to calibrate the photopoint-count to hand-separated botanical composition, we found that for this pasture the surface point count was a direct estimate of tall-growing legume content but overestimated grass and underestimated forb content (Table 2; Fig. 2). There was a relatively low standard deviation (SD) about the regression. When botanical composition was expressed on the basis of total plant material, all components were overestimated, but the SD about the regression did not change (Table 2; Fig. 2). Intercepts not significantly different from 0 (P < 0.05) were removed from the regression. Before removing the intercepts, the  $R^2$  values were 0.92 for the grass and legume fraction of live material and 0.84 for grass, and 0.90 for legume as a fraction of total material. Table 1. Botanical composition of a rotationally stocked pasture expressed as fraction of grass, legume, and forb measured with digital photo point counts and by hand separation of the clipped area, with clipped botanical composition expressed on the basis of live and total plant material.

Grass	Legume	Forb	Dead material		
mean ± confidence interval					
Photo-point-count botanical fractions <sup>†</sup>					
$0.58 \pm 0.11$	$0.30 \pm 0.08$	$0.11 \pm 0.05$	NA		
Hand-separated botanical fraction (live material only)					
$0.51 \pm 0.11$	$0.29 \pm 0.09$	$0.19 \pm 0.06$	NA		
Hand-separated botanical fraction (total live and dead material)					
$0.39 \pm 0.08$	$0.24 \pm 0.08$	$0.16 \pm 0.05$	$0.21 \pm 0.04$		

<sup>†</sup>24 sample areas, 150 points per photo.

Table 2. Calibration regressions between botanical composition measured by hand separation (HS) as a fraction of live and total plant material for grass, legume, and forb components versus their corresponding surface-cover point count (PC) from digital photographs, based on 24 sample areas in the pasture (standard errors reported below the coefficients).

Regression	$R^2$	SDreg <sup>†</sup>
Live plant material		
Grass HS = 0.90 Grass PC	0.98	0.08
0.03		
Legume HS = 1.00 Legume PC	0.97	0.06
0.04		
Forb $HS = 0.11 + 0.73$ Forb PC	0.45	0.10
0.03 0.17		
Total plant material		
Grass HS = 0.67 Grass PC	0.97	0.08
0.02		
Legume HS = 0.85 Legume PC	0.96	0.07
0.04		
Forb $HS = 0.07 + 0.72$ Forb PC	0.57	0.08
0.02 0.13		

<sup>†</sup>Standard deviation about the regression.

### DISCUSSION

Photo point counts of botanical composition are easy to conduct but require cross calibration with paired, handseparated samples to adjust for biases in the point-count method. These biases may be due to differences in the morphology of the species present, including tiller density, leaf angle, and management effects on canopy height and tiller density. For example, forage stands dominated by perennial ryegrass had a lower percentage of white clover dry matter in the stand than did stands dominated by orchardgrass at the same percentage ground cover of clover (Rayburn et al., 2007).

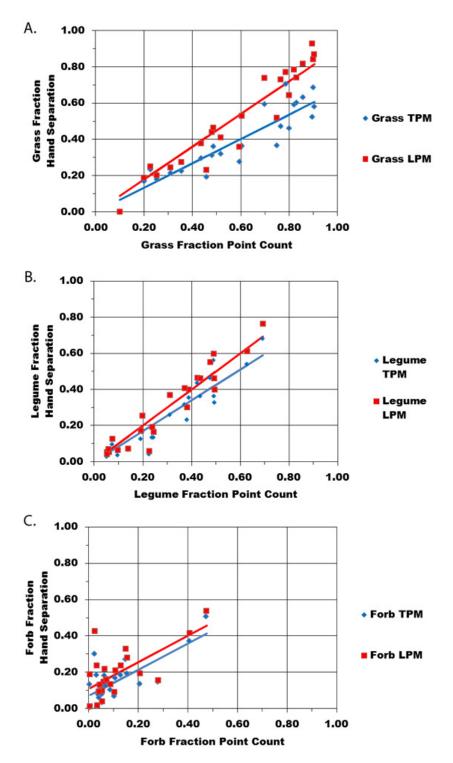


Figure 2. Regressions between pasture botanical composition of grass (A), legume (B), and forb (C) measured by photo point count versus hand separation, expressed as a fraction of total plant material (TPM) and live plant material (LPM).

# Sample Size Required for Point Counts on a Photo

When using point counts within a photo, there is the question of how many points to count to achieve the desired accuracy within each photo. The confidence interval about the measure of a fraction of observations is dependent on the fraction of observations and the number of points counted (Fig. 3). When only 10 points are taken, the confidence interval is very wide. Increasing the number of points to 50 reduces the confidence interval (CI) to less than half that of 10 points, but increasing to 100 points gives only a small improvement in the CI. When pastures have a fractional legume content of 0.2 to 0.5, a pointcount sample size of 100 will provide a CI of about  $\pm 0.10$ (Fig. 3). Since the cost of counting points is relatively low, it may be best to count 100 points or more per photo.

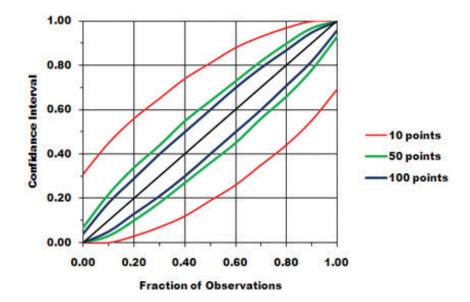


Figure 3. Confidence interval on a binomial distribution fraction observed at three sampling intensities.

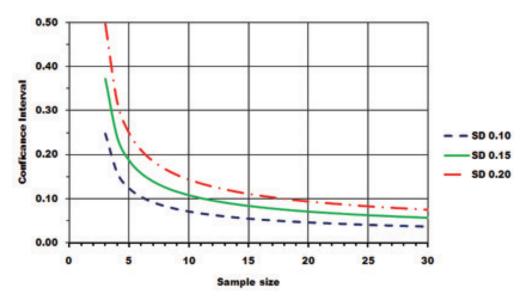


Figure 4. Confidence interval about the sample mean based on sample size and sample standard deviation (SD, P = 0.05) for a normal distribution.

## Sample Size Required for Photos in the Field

The CI about the mean botanical composition in a pasture is determined by the variability of composition at different locations in the pasture as measured using the estimated SD and the number of samples taken across the pasture (Fig. 4). The CI is very wide at a low sample size but decreases greatly as the sample size increases to 10, with a little improvement as the sample size rises to 20. In rotationally stocked, cool-season grass-clover pastures, the SD of pasture legume content averages 0.15 at 30% legume content (Fig. 5). At this level, 12 samples would provide a CI of  $\pm 0.10$ .

### CONCLUSIONS

Hand separation of clipped samples is the standard method for measuring botanical composition in pastures but is very time consuming. The photo-point-count system is a relatively easy, accurate (once calibrated against paired hand-separated samples), and cost-effective alternative. It uses readily available digital cameras and conventional computer software. The photo-point-count data is a direct measure of the canopy surface cover of the botanical component. These point counts can be calibrated to hand-separated dry-matter fraction by using paired clipped, hand-separated samples at a subsample of photo points. For good precision, 12 or more photos per pasture and 100 or more points per photo may be a good balance of in-field and in-photo sampling. For calibration data sets to be used across pastures under the same

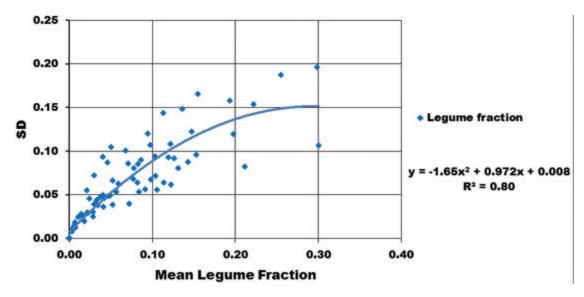


Figure 5. Relation between mean pasture legume fraction and the standard deviation (SD) of the legume fraction for 86 rotationally stocked, cool-season grass–clover pastures.

management and species composition, 12 to 24 paired photo-point-count hand-separated samples across the range of botanical composition present should provide a fairly precise calibration.

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