DIGITAL IMAGE ANALYSIS OF OLD WORLD BLUESTEM COVER TO ESTIMATE CANOPY DEVELOPMENT

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ABSTRACT

Digital image analysis (DIA) can potentially provide rapid, objective assessment of pasture canopy development. In pastures of 'WW-B.Dahl' Old World bluestem [*Bothriochloa bladhii* (Retz) Blake], we compared the DIA programs ImageJ and Canopeo for ground cover estimates, their ability to estimate canopy functions, and their time savings for vegetation analysis relative to a manual method. The DIA procedure involved processing overhead canopy images into two color groupings corresponding to green ground cover and non-green (dead) cover plus bare ground for ground cover calculation. ImageJ analysis of ground cover agreed with two types of Canopeo applications (r^2 values of 0.97 and 0.98). The prediction regression for percentage photosynthetically active radiation interception (PARI) using ImageJ, $y = 0.94x - 2.91$ ($r^2 = 0.72$), was not different from Canopeo. Predicted values of leaf area index (LAI) and biomass increased exponentially with ground cover, likely owing to overlapping leaf area in older canopies. Two-dimensional ground cover had limited power of estimating LAI and biomass when ground cover exceeded 60%, LAI exceeded 1.8, and biomass exceeded 1500 kg DM ha^{-1}. The time required for estimating PARI, LAI, and green biomass using ground cover from both DIA methods was reduced to 3.6% of manual methods. The use of DIA with ImageJ provided measurements of ground cover that are simple and conducive to large batch analyses. Regressions of ground cover were deemed useful for rapid estimations of PARI and LAI, but of lesser value for biomass, especially when canopies developed stems and seedheads.

Core Ideas

- ImageJ and Canopeo agreed in accurately measuring vegetative ground cover of pasture.
- Light interception was linearly related to ground cover measurement with ImageJ and Canopeo.
- ImageJ and Canopeo reduced the time for estimating canopy development by 96%.

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Accurate, low-labor assessment of ground cover and canopy development of grazing land is important for managing sustainable animal stocking rates for assuring forage supply and soil protection from erosion. Adequate vegetative cover confers resistance to erosion by wind and water, by preventing soil particle detachment and mitigating raindrop-impact effects on soil disaggregation (Troeh et al., 2004). Techniques for rapid assessment of pasture canopies may also aid forage researchers in controlling forage allowance in grazing trials (Baxter et al., 2017b), modeling plant growth (Patrignani and Ochsner, 2015), and assessing cover crops (Büchi et al., 2018). Interception of photosynthetically active radiation (PAR) by the plant canopy is essential for biomass productivity, and is a function of the canopy leaf area (Nelson, 1996). Radiation use efficiency, the ability of a canopy to convert intercepted PAR into dry matter growth, is widely used in crop simulation models to drive the accumulation of plant biomass based on development of the LAI and the fraction of PAR interception (PARI) (Kiniry et al., 2005). Accurate measurements of LAI and PARI are fundamental for determining the proper radiation use efficiency for a particular crop over the expected range of growing conditions. Manual measurements of PARI and LAI are time and labor intensive, and conventional LAI measurements are destructive of plant canopies.

Quantifying canopy cover with digital image analysis (DIA) offers a potential method to rapidly and nondestructively estimate LAI, PARI, and biomass. Digital image analysis entails capturing a two-dimensional, overhead image of the canopy with a digital camera, followed by calculation using DIA software to determine the percentage of green pixels associated with functional photosynthetic areas. Canopy cover using DIA was measured in bermudagrass [*Cynodon dactylon* (L.) Pers.] turf (Richardson et al., 2001) and in soybean [*Glycine max* (L.) Merr.] (Purcell, 2000) using inexpensive digital cameras and SigmaScan Pro software (SPSS, Inc., Chicago, IL). Purcell (2000) reported a strong relationship ($r = 0.97$) between fractional canopy cover and fractional PARI using DIA. Compared with conventional measurements of LAI, PARI, and biomass, DIA techniques could allow greater number of field sampling

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Abbreviations: DIA, digital image analysis; LAI, leaf area index; OWB, 'WW-B.Dahl' Old World bluestem; PAR, photosynthetically active radiation; PARI, photosynthetically active radiation interception; RMSE, root mean square error.

sites, reduce time and labor requirements, and provide nondestructive measurements of vegetative cover that are more objective than visual estimates.

There is recent progress in developing application software that permits rapid, user-friendly measurements of canopy characteristics for pasture management and research. Patrignani and Ochsner (2015) developed Canopeo and tested its accuracy and speed in relation to other DIA methods for short and tall grasses using Matlab programming language (Mathworks, Inc., Natick, MA). Canopeo is available (http://www.canopeoapp.com) for analysis of images using the downloadable "Canopeo Matlab app," which can process batches of pre-captured images for green canopy cover. The authors also developed a smartphone app on Android and iOS platforms, which captures a single image with the built-in camera, and calculates an instant measurement of green canopy cover. Büchi et al. (2018) compared the use of Canopeo to visual estimates of ground cover of 20 cover crop species and two mixtures. They concluded that visual estimates usually understated actual ground cover as determined with Canopeo, especially at intermediate levels of ground cover of narrow-leaved crops. Chung et al. (2017) used Canopeo as a rapid measurement of biomass of sorghum (*Sorghum bicolor* Moench.) plants by recording images of vertical growth rather than ground cover.

ImageJ is a freely available (https://imagej.nih.gov/ij/) DIA program that was developed originally for medical research (Schindelin et al., 2015). It has full-function color and gray-scale processing abilities for any image format (Ferreira and Rasband, 2012). Baxter et al. (2017b) published a macro code for rapid processing of large numbers of images. The accuracy of ImageJ for estimating green canopy cover in perennial pastures has not been evaluated. Baxter et al. (2017a) compared ImageJ to other canopy assessment techniques for percentage alfalfa (*Medicago sativa* L.) content (dry weight basis) when grown with either WW-B.Dahl Old World bluestem (OWB) or tall wheatgrass [*Thinopyrum ponticum* (Host) Beauv.]. Greater predictive ability of alfalfa content was attained when grown with OWB (R^2 = 0.40 vs. 0.08 with tall wheatgrass), which they attributed to a greater differential in green hue between grass and alfalfa than in the mixture with tall wheatgrass. The predictive ability with ImageJ for alfalfa content in OWB was considered low compared with visual estimates (R^2 = 0.76 for visual estimates), owing to the limitation of a two-dimensional image to account for three-dimensional factors that define species content on a mass basis, such as overlapping of leaves at high LAI.

We hypothesize that ImageJ can provide canopy cover assessments of OWB that compare favorably with those of Canopeo, and provide rapid estimates of canopy cover that predict LAI, PARI, and biomass of OWB. This warm-season grass was selected because of its favorable adaptation to low-water supply in the southern Great Plains and its distinctive periods of vegetative and reproductive stages in early and late summer, respectively (Dewald et al., 1995). The objectives of this study were to (i) test the agreement between ImageJ and Canopeo for quantifying vegetative ground cover via DIA; (ii) determine the predictive ability of using canopy cover derived from ImageJ to estimate LAI, PARI, and biomass of OWB grass; and (iii) compare the time requirements for estimating LAI, PARI, and biomass using manual techniques vs. use of DIA by ImageJ and Canopeo.

METHODS AND MATERIALS

Site Description

This study was conducted on established pastures of OWB at the Texas Tech Forage-Livestock Research Laboratory located 10 km east of New Deal in northeast Lubbock County (101°47′ W, 33°45′ N; 993 m elevation). Soil and pasture characteristics including management history are detailed by Baxter et al. (2017b). The forage canopy was sampled in each growing season of 2013 to 2016 during two growth periods, from approximately mid-May to mid-July and mid- or late July to early October. All fields in this study were ungrazed, and hay was produced at the end of the first growth period by mowing to 8-cm stubble height. Sampling of the regrowth during the second period terminated when the stand attained the mature seed stage. Fields were irrigated with subsurface drip lines (Netafim USA, Fresno, CA) at 0.36-m depth and 1-m spacing. Amounts of irrigation and rainfall varied by year with the intent of not supplying more than 229 mm of irrigation per year.

Field Sampling

Data collection in 2013 to 2016 consisted of taking photographic images and canopy measurements biweekly in randomly located quadrats in each field within 1 h of the local solar noon (1237 h). The sampling area was defined by a 1-m by 1-m PVC frame quadrat. There were 12 quadrat plots in 2013 per sampling date, which occurred in only one pasture replication. In 2014 through 2016, there were 10 quadrats in each of three pasture replications for a total of 30 quadrats per sampling date. Weeds inside each quadrat were sparse and were hand-removed before any imaging and measurements. Canopy cover images were recorded from 1.5-m height by an Olympus TG-820 (Olympus Optical Co., London, UK) waterproof, dust-proof digital camera mounted on a PVC monopod (Baxter et al., 2017a). Images were saved as JPEG format at 1280 by 960 pixels. The camera was leveled with respect to the ground, and the monopod was tilted so that the camera was centered over the quadrat. The images were recorded on the camera's internal memory card, and transferred to an office computer for subsequent analysis.

A Li-Cor LI-1400 1-m line quantum sensor (Li-Cor, Inc., Lincoln, NE) was used to measure PAR. Duplicate readings of irradiance were recorded when the sensor was held level above the canopy, and when the sensor was placed at soil level under the canopy. The duplicate readings were recorded at right angles to each other, and averaged for each quadrat. Then PARI was calculated and expressed as a percentage.

$$\text{PARI} = (1 - \text{PAR beneath canopy}/\text{PAR above canopy}) \times 100$$

A subsample of forage biomass was clipped at 8-cm stubble height in each quadrat, sealed in a zipper-locked polyethylene bag, and stored at 4°C. The remainder forage biomass was clipped at 8 cm, placed in a paper bag, and dried in a forced-air oven at 55°C. The subsamples were separated into green leaves and stems apart from the senesced tissues. Green tissues of subsamples were measured for leaf area by a Li-Cor LI-3100 area meter (Li-Cor, Inc., Lincoln, NE) while the leaves were still hydrated, and then dried at 55°C. Senesced tissues were

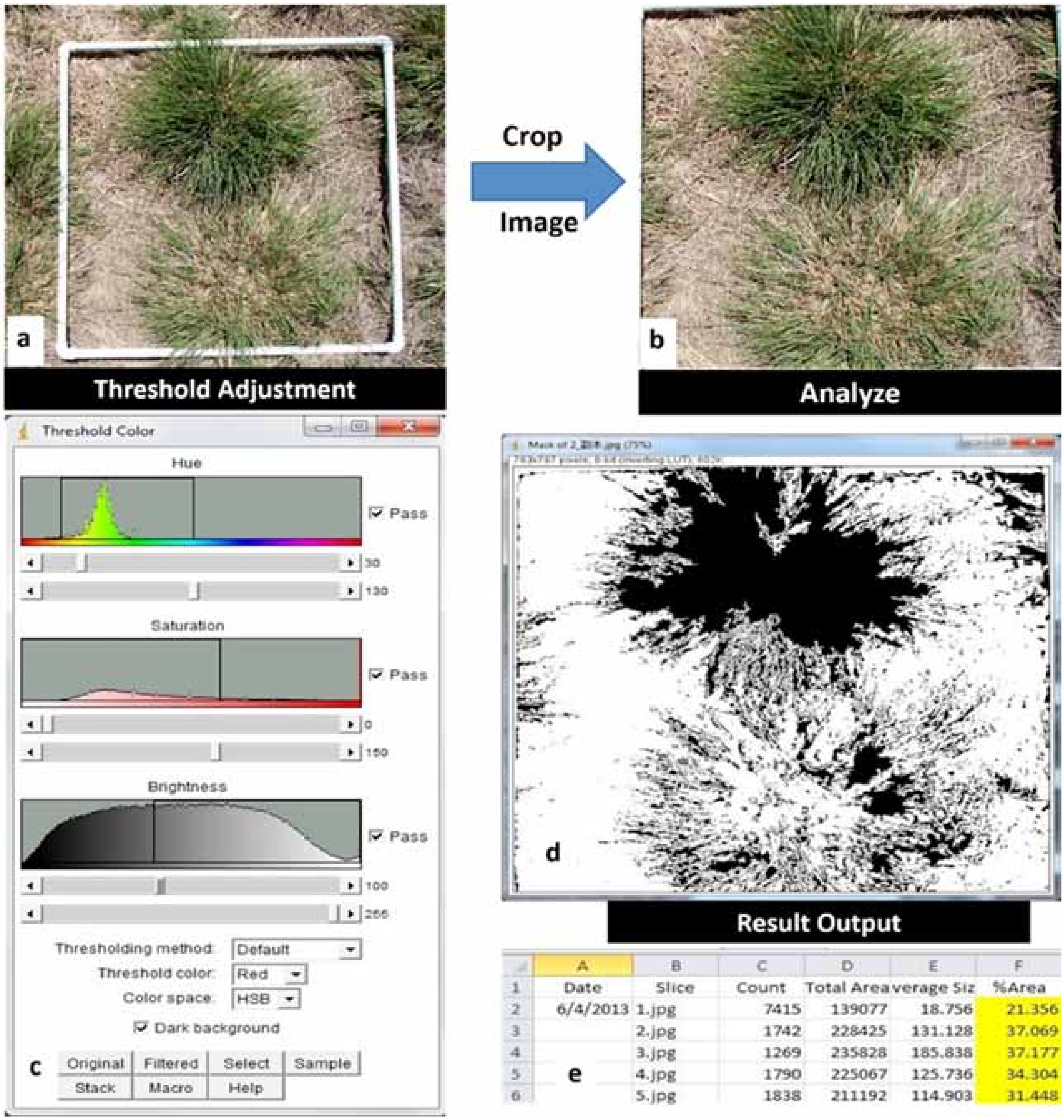

Fig. 1. Digital image analysis process from ImageJ: (a) raw image capture; (b) image input in ImageJ; (c) ImageJ threshold settings; (d) green tissue in the image is selected; (e) green tissue is re-selected by "mask" function when analyzing the selected pixels.

oven dried and weighed separately to calculate the fraction of senesced mass. The mass of the dried subsamples (green + senesced) was added to the remainder mass to determine total mass per quadrat. The fraction of dry, senesced material to total biomass in the subsample was used to correct the total biomass yield to green mass yield. Leaf area index (m^2 m^{-2} or unitless) was calculated as the product of *a*, the specific leaf area (ratio of green leaf area to green leaf mass) of the subsample; *b*, the fraction of total leaf mass that was green; and *c*, the total leaf mass (green + senesced) of the quadrat; thus LAI = $a \times b \times c$.

Digital Image Analysis

Photographic image files were transferred to computer folders on returning from the field. Images were cropped to represent the exact 1-m^2 area, and saved with a new name, thereby always allowing retrieval of the original image. The canopy images were processed individually by using ImageJ (Version 1.49b). Image J was able to separate the image into two portions: green and non-green areas (senesced leaves, ground litter, and bare ground) by adjusting hue, saturation, and brightness threshold settings to match the range of observed green (live tissue) colors. Previous studies using SigmaScan for image analysis (Karcher and Richardson, 2005; Richardson et al., 2001) conducted on turf-type bermudagrass suggested hue and saturation adjustments based on results matching with preset turf-cover percentages, with no brightness adjustments needed because of the use of a closed light box. The hue, saturation, and brightness settings in ImageJ range from 0 to 255. Preliminary work with images of OWB indicated that a hue range from 30 to 130 and a saturation range from 0 to 150, with brightness adjusted depending on specific sampling date (no light box used). These settings matched the range of actual green colors of the target live leaves (Fig. 1a–c).

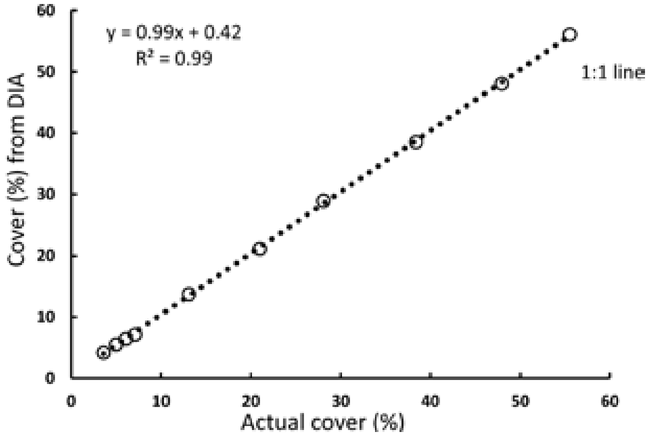

Fig. 2. Regression of predicted values of cover using digital image analysis (DIA) by ImageJ vs. actual cover of paper strips on a paper background, in comparison with a 1:1 line.

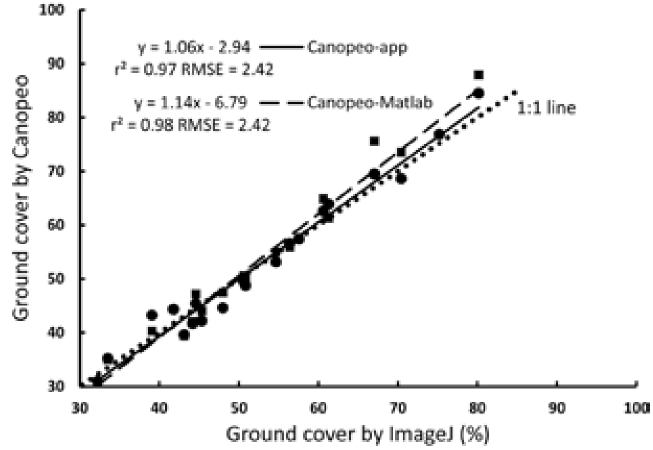

Fig. 3. Regression of ground cover estimated by Canopeo-app or Canopeo-Matlab on ground cover by ImageJ in comparison with a 1:1 line. Data were collected in 2018 with 20 total sampling points across three paddocks.

As the growth periods progressed, selecting green tissue became harder because canopy growth caused overlapping and shading of leaf blades. The "Mask" function was then used to display filled outlines of the measured particles, thereby improving the selection of shaded green areas that were deeper in the canopy (compare Fig. 1c and d). Following the ImageJ user guide (Ferreira and Rasband, 2012), selected image particles were reselected and analyzed by the following operations: select "Analyze > Analyze Particles." According to the user guide, after the manual window popped out, we selected "Show: > Masks," clicked "Display results," "summarized" and "include holes" boxes, then checked "OK" for the software to analyze the particles. The images were reselected as shown in Fig. 1e, and the analyzed results were saved as a Microsoft Excel worksheet (.xlsx format) or Microsoft Excel Comma Separated Values File (.csv format). The macro code developed by Baxter et al. (2017b, Appendix) was used to batch-process all images captured on a single sampling date. The same brightness setting was applied to all images within a batch to represent the same light conditions.

A test of the accuracy of the camera and ImageJ to measure areas of long, narrow objects was conducted in the laboratory by creating long and narrow strips of construction paper of premeasured areas. There were two colors of paper, standard black (commercially cut to 57 cm by 71 cm) for the background to simulate bare ground, and white for the strips serving as simulated leaves. The white strips were manually cut into varying dimensions of 5 to 12 cm by 0.3 to 1 cm and laid onto the background in random, non-overlapping arrangements. Ten arrangements were made of the strips, varying from 2 to 15 pieces such that the exact total area of the strips, and thus its percentage on the black background, was known from dimensional measurements. Photographic images were taken of each arrangement, and percentage cover of the simulated leaves was calculated by DIA using ImageJ. Linear regression of DIA values of cover on actual percentage cover was performed and compared to an ideal 1:1 function using SAS (SAS Institute Inc., Cary, NC).

The Canopeo-Matlab application was downloaded to a microcomputer using the Windows 10 operating system (Microsoft, Inc., Redmon, WA). The application was installed into the preloaded Matlab software. Within the application, the same images that were used by ImageJ analysis were analyzed by the default setting in Canopeo based on the color threshold criteria. The results were saved in a .txt file, and later transferred to a Microsoft Excel worksheet (.xlsx format) for future analysis. The Canopeo phone app was downloaded to an iOS iPhone 7Plus (Apple, Inc., Cupertino, CA). New images were captured over the same sampling plots by the iPhone camera and then immediately analyzed. The Canopeo phone app lacks the means for processing a large batch of images; however, the process time was less than 10 s per image. The resulting screen produces a binary image with white pixels directed by the built-in, default color threshold criteria, which are adjustable by a slider bar. The resulting cover percentage is saved into a group folder in the application. Outputs of data files can be downloaded from the Canopeo website into Microsoft Excel (.csv format), or manually inputted into a Microsoft Excel worksheet (.xlsx format). In the Canopeo-Matlab, a noise reduction function allows one to include isolated green pixels that were not accepted by the default setting criteria. This is to verify that the long, narrow grass leaves are captured and digitized. The color threshold criteria were set for all images captured within a sampling date for the computer and phone image analyses. Relationships between each of the Canopeo methods (Canopeo-Matlab and Canopeo-app) vs. ImageJ for ground cover were analyzed for agreement with the 1:1 standard using linear regression in SAS.

The duration of each task involved in determining PARI, LAI, and biomass yield using the traditional manual method and DIA was logged to quantify the labor investment for each measurement. Results are expressed as person-minutes (person-min) per one square meter sampling area. This comparison was not set up with replicated statistical analysis, but rather to inform users of the potential time requirements for collecting such data. Prediction functions for PARI, LAI and biomass were derived with linear and nonlinear regression analyses on ground cover estimated by ImageJ and Canopeo-Matlab as the independent variable using SAS.

RESULTS AND DISCUSSION

The percentage cover by simulated leaves from DIA using paper strips was estimated accurately in comparison with the actual cover values (Fig. 2), as shown by regression having a

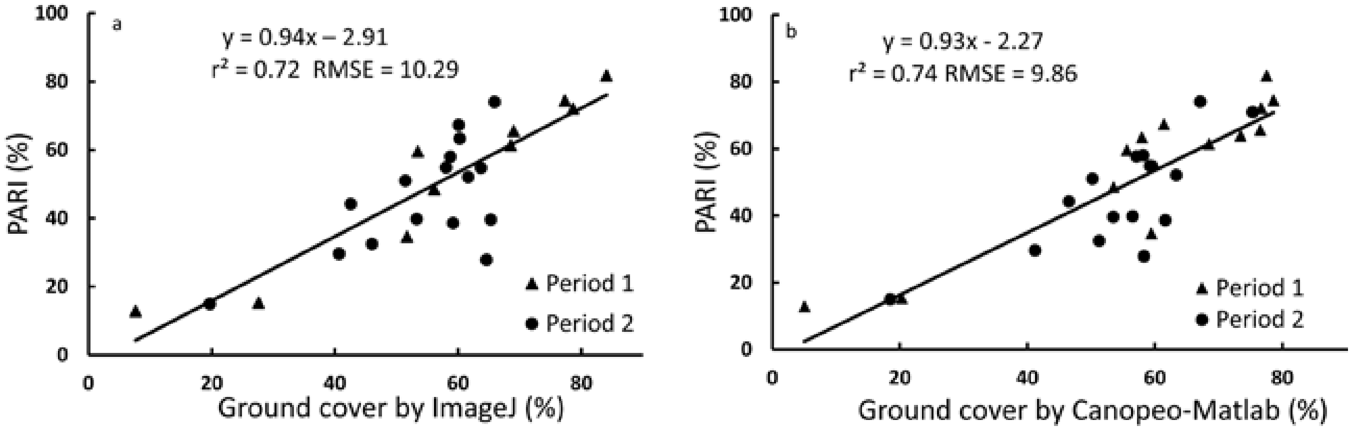

Fig. 4. Photosynthetically active radiation interception (PARI) vs. percentage ground cover by (a) ImageJ and by (b) Canopeo-Matlab. Points represent sampling dates over 4 yr, and are means of 12 observations in 2013 and 30 observations per year in 2014 to 2016. Samplings occurred during late spring to early summer (Period 1) and late summer to early fall (Period 2).

high coefficient of determination (R^2 = 0.99), with slope (0.99) not different from 1.0 and *y*-intercept (0.42) not different from zero (P = 0.99 and P = 0.89, respectively). This supported the accuracy of using a digital image from a camera and the ImageJ software to calculate two-dimensional cover of a targeted color against a contrasting background color.

The two relationships between Canopeo and ImageJ differentiate between the phone app and Canopeo-Matlab versions (Fig. 3). The phone app showed a strong linear regression (r^2 = 0.97) with slope not different from 1.0 (P = 0.18) and *y*-intercept not different from zero (P = 0.07). This close agreement with the 1:1 line occurred with the phone and camera devices recording independent images as opposed to loading the phone-app images into ImageJ. When comparing Canopeo-Matlab with ImageJ (Fig. 3), a similar high r^2 value was revealed; however, the slope of 1.14 was greater than 1.0 (P = 0.003) and *y*-intercept differed from zero (P = 0.010). The slope greater than 1.0 resulted in cover estimates from Canopeo-Matlab exceeding those of ImageJ at the high end of the data range.

ImageJ and Canopeo (Matlab and phone apps) are free applications for all users; however, the Canopeo computer application requires the Matlab software, which is currently US$500 for the license. The Canopeo phone app is easy to learn and only requires a smart-phone device. Users have very little control of the image processing to correct for different hues of green, degrees of senescence, and light conditions. ImageJ requires basic set-up of the program and minimal training on adjusting color thresholds. ImageJ and Canopeo-Matlab are good choices for researchers who process large numbers of images. Color thresholds in ImageJ are done with immediate visual feedback to the user, whereas in Canopeo-Matlab, visual feedback is afforded via the Preview button. Saving and exporting processed images and calculated cover data from the Canopeo phone app required several manual steps, whereas data transfer involved fewer, simpler steps with ImageJ and Canopeo-Matlab.

Photosynthetically active radiation interception was linearly related to ground cover estimated by ImageJ (Fig. 4a) and Canopeo-Matlab (Fig. 4b) with slope not different from 1.0 and *y*-intercept not different from zero (P = 0.70 and P = 0.66, respectively) indicating a 1:1 relationship with ground cover for both methods. This relationship suggests that overlapping of leaves at high levels of ground cover did not underestimate PARI in relation to the simple two-dimensional recording of canopy cover. The data points for both periods were expressed by the same regression, but with fewer points in Period 2 at the high end of cover. The regressions suggest that rapid and fairly accurate estimates of PARI are possible using DIA.

Leaf area index was exponentially related to ground cover as measured by ImageJ (R^2 = 0.88, Fig. 5a) and Canopeo-Matlab (R^2 = 0.84, Fig. 5b). There was no difference in exponent estimation (P = 0.94) between two methods. Both regressions show that LAI increased gradually to 1.8 over large increases in cover from 8 to 66%, followed by large increases in LAI when cover exceeded 66% (Fig. 5). All but one of the data points above 66% cover were observations from Period 1, when the number of emerging reproductive stems was low, but there were large amounts of overlapping leaves. Greater stem and seedhead elongation was observed to occur during Period 2. At 10 yr after establishment, OWB had large crowns with frequent spaces between crowns. We observed that during early season canopy development, tillers at the perimeter of the crowns tended to grow out from the crown edge with leaf angle perpendicular to the sun and covering bare ground. Later-season canopy development involved somewhat more-erect leaf appearance, but with greater variety of leaf angles and still considerable leaf overlap on later dates.

Biomass production had an exponential relationship to ground cover estimated by ImageJ (Fig. 6a) and Canopeo-Matlab (Fig. 6b). There was no difference in exponent estimation (P = 0.89) between the two methods. Biomass increased strongly and with high variability when ground cover exceeded 55% and biomass exceeded 1500 kg ha^{-1}. As with LAI (Fig. 5) the fitted plot curved upward as cover increased (Fig. 6), but with lower R^2, indicating poorer ability of ground cover to estimate biomass. The curvilinear relationship reinforced the fact that ground cover, as a two-dimensional metric, could not linearly represent the three-dimensional pattern of biomass accumulation, especially during reproductive stem formation. This result agreed with Baxter et al. (2017a) that DIA was ineffective in predicting forage availability when stems elongated and seedheads formed. ImageJ was incapable of predicting alfalfa–tall wheatgrass biomass when tall wheatgrass was in the reproductive phrase, and ImageJ was ineffective in distinguishing alfalfa cover from grass cover because of similar deepness of

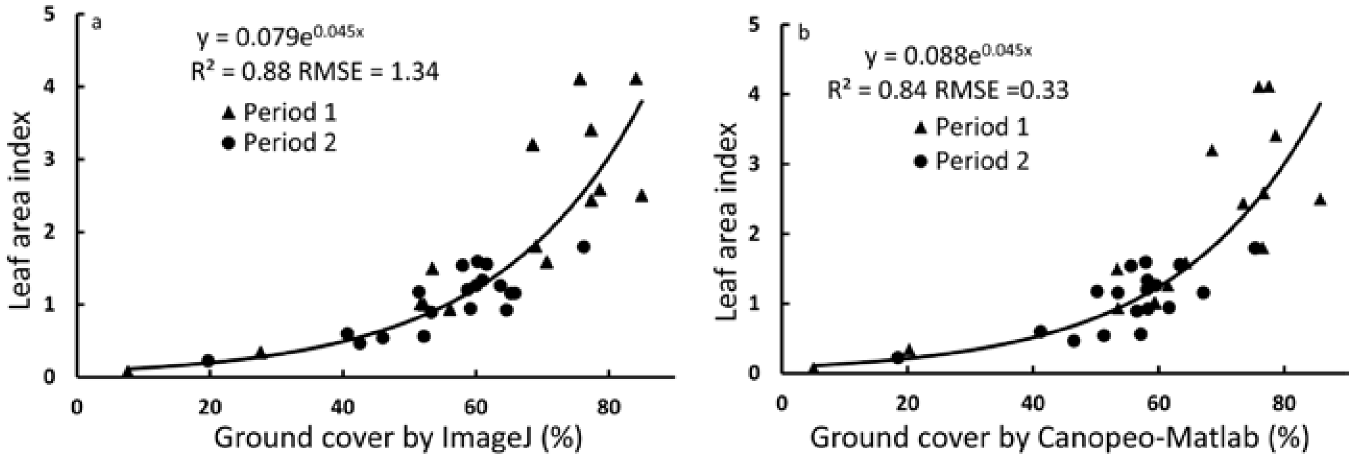

Fig. 5. Leaf area index vs. percentage ground cover estimated by (a) ImageJ and (b) Canopeo-Matlab. Points represent sampling dates over 4 yr, and are means of 12 observations in 2013 and 30 observations per year in 2014 to 2016. Samplings occurred during late spring to early summer (Period 1) and late summer to early fall (Period 2). The function is linearized as (a) $\ln(y) = 0.045x - 2.54$ (RMSE = 0.29); (b) $\ln(y) = 0.045x - 2.47$ (RMSE = 0.31).

the green hues of the two species (Baxter et al., 2017b). In analysis of vertical images of canopies using Canopeo-app, Chung et al. (2017) obtained predictions of sorghum biomass yield with higher correlations than we observed with OWB, which would be appropriate for an upright-growing crop. Supplementing the overhead, two-dimensional imagery in our study with vertical-canopy imagery would have been impractical owing to the largely prostrate orientation of grazed canopies.

Our research found that percentage PARI and LAI were fairly well predicted by ground cover when OWB exhibited predominantly leafy growth. Leaf overlaps and seedhead production in the latter part of growth periods introduced high variability and lessened the ability of ground cover to predict biomass.

Manual methods of measuring PARI, LAI, and biomass required a mean of 61 person-min to derive the results per 1-m^2 sampling plot (Table 1), not including the time for oven drying. By contrast, DIA from ImageJ for canopy cover and then using predictive equations to calculate PARI, LAI, and biomass (regressions explained below) took 2.2 person-min per sample, or 3.6% of the time required for the manual measurements. Similarly, DIA with Canopeo reduced the person-min to 3.9% of manual measurements. The manual PARI measurement required two persons for PAR readings, including two readings above and two below the canopy, followed by calculation of interception. After the camera was mounted and leveled on the monopod, image recording of a sample plot took less than 0.2 min by one person. Labor requirement for biomass clipping varied throughout the season, but it took an average of 20 person-min to clip and bag a 1-m^2 quadrat, and 0.2 min to handle, weigh, and record each dried sample. Leaf area measurement took 45 person-min per sample, including time to bag, weigh, and calculate LAI. Individual image processing in ImageJ, which needed only one person, required 0.5 person-min including importing the image, adjusting settings, and analyzing the image via the macro. Processing time per image is reduced as the number of images in an analysis batch increases. In this study, the average handling time of one batch of 36 images was 0.5 min in ImageJ. There was negligible difference between Canopeo-Matlab (0.2 s) and ImageJ (0.5 s) for batch canopy analysis, therefore the time savings on batch analysis between the DIA methods are comparable. The Canopeo phone app took only 1.0 person-min to record and process an individual image. However, the phone app

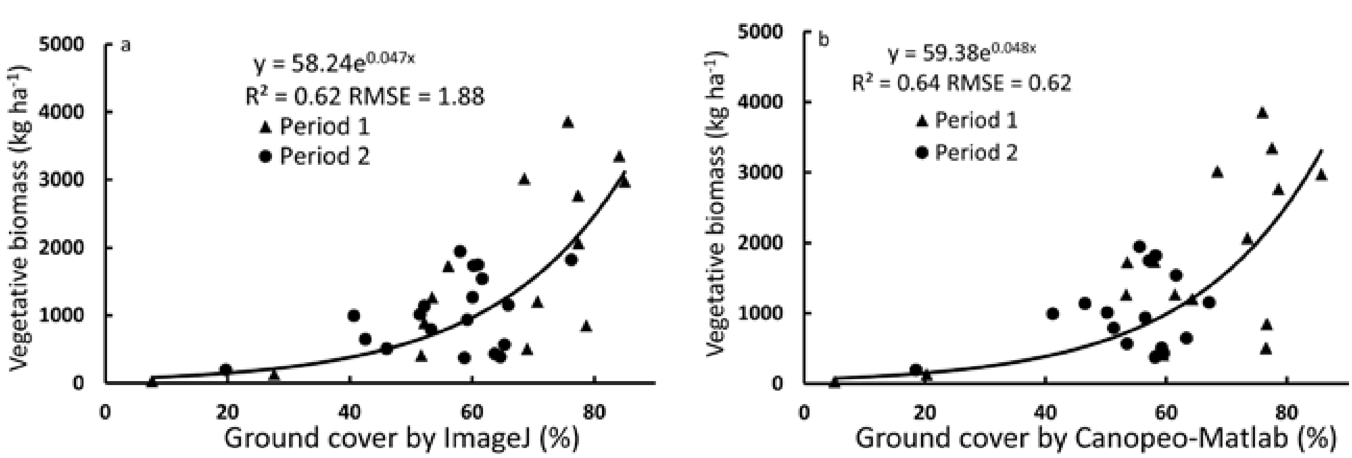

Fig. 6. Biomass vs. percentage ground cover estimated by (a) ImageJ, and by (b) Canopeo-Matlab. Points represent sampling dates over 4 yr, and are means of 12 observations in 2013 and 30 observations per year in 2014 to 2016. Samplings occurred during late spring to early summer (Period 1) and late summer to early fall (Period 2). The function is linearized as (a) $\ln(y) = 0.047x + 4.06$ (RMSE = 0.63); (b) $\ln(y) = 0.048x + 4.01$ (RMSE = 0.66).

Table 1. Comparison of labor requirement as person-minutes per sampling plot for manual (traditional) and digital image analysis (DIA) methods of collecting data on canopy variables.

Manual†		DIA by ImageJ‡		DIA by Canopeo app§		DIA by Canopeo Matlab¶	
Task	Labor	Task	Labor	Task	Labor	Task	Labor
			person-min				
PAR interception	5.0	Recording image	0.2	Recording image	0.2	Recording image	0.2
Leaf area index	45.0	Image loading	0.5	Image loading	0	Image loading	0.5
Forage biomass	10.0	ImageJ analysis	0.5	Canopeo analysis	0.8	Canopeo analysis	0.5
Canopy regressions	1.0	Canopy regressions	1.0	Canopy regressions	1.5	Canopy regressions	1.2
Total	61.0	Total	2.2	Total	2.5	Total	2.3

† Includes field measurement of photosynthetically active radiation (PAR), clipping, transport to ovens, separating live from dead tissue, feeding leaf area meter, handling and weighing dried samples, but not drying time.

‡ Includes mounting camera, recording image, transferring to a computer, and image analysis.

§ Includes mounting phone camera, recording image, image analysis, and exporting results.

¶ Includes mounting camera, recording image, transferring to a computer, image analysis, and exporting results.

does not allow batch analysis, thus scaling up to large numbers of images does not reduce per-sample analysis time unless images are aggregated and uploaded to the computer program.

SUMMARY AND CONCLUSIONS

Digital image analysis is an accurate, nondestructive, and low-labor method to evaluate ground cover in OWB grassland. Analysis of ground cover using ImageJ was highly positively correlated with both versions of Canopeo (Matlab and phone app) and produced close fits to a 1:1 relationship. Our results support the Canopeo phone app as a rapid, accurate tool for pasture managers and consultants to make on-site measurements of ground cover to aid in monitoring pasture growth and condition. Ground cover as measured with DIA by ImageJ and Canopeo-Matlab both produced significant linear functions for predicting PARI. Regressions for predicting LAI and forage biomass from DIA-generated ground cover produced exponential functions, likely reflecting increased overlapping of leaves and stems in later stages of canopy development. The exponential functions suggested reduced power of estimating LAI and biomass as ground cover exceeded 60%, LAI exceeded 1.8, and biomass exceeded 1500 kg DM ha^{-1}. Regressions of ground cover were deemed useful for rapid estimations of PARI and LAI, but of lesser value for biomass, which had a lower coefficient of determination. The labor requirement to estimate PARI, LAI, and forage biomass based on DIA-calculated ground cover was 2.2 person-min, constituting a 96% reduction in labor compared with traditional manual methods (61 person-min).

Digital photography and image analysis offer a low-labor means of collecting accurate ground-cover data from grass pastures. The technique substantially saves time and labor, is more objective than visual estimates, allows large numbers of images to be recorded, processed, and archived, reduces the tedium of manual measurements, and avoids destruction of the plant canopy. High correlations of the Canopeo applications with ImageJ for ground cover suggest that both types of DIA programs are useful in monitoring PARI and LAI. ImageJ does not require the Matlab software to process large batches of image analyses.

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