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Canopeo: A Powerful New Tool for Measuring Fractional Green Canopy Cover

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ABSTRACT

Fractional green canopy cover (FGCC) is a key diagnostic variable that can be used to estimate canopy development, light interception, and evapotranspiration partitioning. Available image analysis tools for quantifying FGCC are time-consuming or expensive and cannot analyze video. Our objective was to develop a simple, accurate, and rapid tool to analyze FGCC from images and videos. This tool, called Canopeo, was developed using Matlab and is based on color ratios of red to green (R/G) and blue to green (B/G) and an excess green index (2G–R–B). The output from this tool was compared to that from two software packages widely used to analyze FGCC, SamplePoint, and SigmaScan Pro. Canopeo's image processing speed was 20 to 130 times faster than SigmaScan and 75 to 2500 times faster than SamplePoint. Canopeo correctly classified 90% of pixels when compared to SamplePoint. Root mean squared difference (RMSD) values for Canopeo FGCC vs. FGCC determined by SamplePoint and SigmaScan ranged from 0.04 to 0.12, with an average RMSD of 0.073 across several sets of images of corn (*Zea mays* L.), forage sorghum [*Sorghum bicolor* (L.) Moench], bermuda grass [*Cynodon dactylon* (L.) Pers.], and switchgrass (*Panicum virgatum* L.). Analysis of video recordings of transects over crop canopies proved to be useful to minimize sampling error and to quantify FGCC spatial variability. This analysis was simple and rapid with Canopeo but not possible with SamplePoint or SigmaScan. The Canopeo app for Matlab and for iOS and Android mobile devices can be downloaded at www.canopeoapp.com.

Active plant canopies play an important role in the Earth's atmospheric dynamics, surface energy balance, and soil water balance (Wittich and Hansing, 1995). To measure the extent of canopy development numerous indices such as spectral vegetation indices, leaf area index (LAI), and FGCC have been developed. Fractional green canopy cover has emerged as a nondestructive and relatively easy-to-measure variable that is employed in disciplines such as ecology, environmental science, and agronomy to quantify active vegetative land cover at different scales in space and time. For instance, FGCC was used to measure forest land cover of Scots pine (*Pinus sylvestris* L.) and Norway spruce [*Picea abies* (L.) Karst] by Korhonen et al. (2006). Also, a relationship between FGCC and light interception was developed to estimate the proportion of green and senescing leaves in soybean [*Glycine max* (L.) Merr.] (Purcell, 2000). The use of FGCC has also been examined to measure the color and percent land cover in turf (Karcher and Richardson, 2003; Richardson et al., 2001), and to measure growth rate of weeds after tillage events (Rasmussen et al., 2010). Sharma and Ritchie (2015) used FGCC along with crop height and normalized difference vegetation index (NDVI) to monitor cotton (*Gossypium hirsutum* L.) growth under different irrigation regimes in high-throughput phenotyping studies in Texas. Statistical correlations between FGCC, LAI, NDVI, and aboveground biomass have also been developed by multiple researchers (Carlson and Ripley, 1997; Lati et al., 2011; Lukina et al., 1999; Nielsen et al., 2012; Rundquist, 2002). Crop FGCC is a key variable in soil-plant-atmosphere models such as Aquacrop (Hsiao et al., 2009; Raes et al., 2009; Steduto et al., 2009), in which FGCC is used to estimate crop water use.

Canopy cover has traditionally been measured using subjective methods (Richardson et al., 2001; Robson et al., 2013). The projection of images on a grid or transparency for point classification helped to reduce subjectivity, but these methods are not efficient when analyzing large sets of images (Corak et al., 1993; Ribeiro et al., 2011). In recent decades, improvements in the quality of images produced by affordable digital cameras and mobile devices propelled the use of digital images for FGCC measurements. At the same time, numerous image processing methods (Behrens and Diepenbrock, 2006;

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Abbreviations: ACT, automatic color threshold; B/G, blue to green; FGCC, fractional green canopy cover; LAI, leaf area index; MPC, manual pixel classification; NDVI, normalized difference vegetation index; R/G, red to green; RMSD, root mean squared difference.

Shrestha and Steward, 2003; Thorp and Dierig, 2011; Thorp et al., 2008) and software packages (Ewing and Horton, 1999; Purcell, 2000; Rasmussen et al., 2007) have been developed to analyze digital images of plants for a variety of applications.

Currently available green canopy cover software packages can be classified in two groups according to their pixel classification principle: manual pixel classification (MPC) and automatic color threshold (ACT) classification. The first type uses a set of randomly selected pixels (usually less than 250 random points), which have to be manually classified by a trained user. The manual classification of pixels has high accuracy, but it is time consuming because the user typically classifies 100 to 250 pixels per image. Sampling error is also a concern with MPC methods because only a miniscule fraction of the millions of pixels in a typical image are actually classified. Manual pixel classification methods are particularly useful when calibrating ACT methods (Booth et al., 2006) or when calculating the proportion of various plant species or other components such as plant residue, soil, or rocks that are not easily distinguishable using color threshold settings. An example of a widely used MPC product is SamplePoint, a program developed by Booth et al. (2006). Applications of SamplePoint have included monitoring ground cover in cropping systems research (Krueger et al., 2012), monitoring plant phenology (Crimmins and Crimmins, 2008), studying grazing intensity and spatial variability in grasslands (Augustine et al., 2012), and developing quantitative relationships between FGCC and LAI in crops so that LAI data can be used in the Aquacrop simulation model (Nielsen et al., 2012).

The ACT type of software requires the specification of color thresholds or color ratios to select the desired portion of the image. This type of software is advantageous because a computer does the pixel classification, and therefore image processing time is markedly reduced. Another benefit of ACT methods is that all pixels in the image are classified. However, undesired pixels may be selected, leading to under or over estimation of the variable of interest. An example of a widely used ACT software package in agronomy is SigmaScan Pro 5, a product of Systat software (Chicago, IL). This software requires user-specified hue (range from 0–360) and saturation values (range from 0–100) (Purcell, 2000). This software has been used to analyze canopy cover and light interception in soybean (Purcell, 2000), percent turf coverage (Karcher and Richardson, 2005; Richardson et al., 2001), and turf color (Karcher and Richardson, 2003). Even though this software can be significantly faster than SamplePoint, high resolution images can result in processing times >30 s per image. Traditionally, a small number of images have been taken to represent research plots or experimental fields, however, available technology is generating a growing interest for high spatial and temporal monitoring of plant growth, generating large datasets that require faster image processing tools. In addition, the user cannot visualize the effects of the chosen threshold values before batch image processing in SigmaScan, nor can SigmaScan analyze video recordings.

Given the limitations of current software to quantify FGCC, there is a need to develop new tools that overcome those limitations and provide convenient and accurate methods to analyze FGCC. The objectives of this study were (i) to develop an interactive, simple, and accurate tool capable of rapidly analyzing high resolution digital images and video recordings to quantify FGCC, and (ii) to evaluate the accuracy and image processing speed of that tool relative to two software packages widely used in agronomic research.

MATERIALS AND METHODS

Canopeo Description

Canopeo is an ACT image analysis tool developed in the Matlab programming language (Mathworks, Inc., Natick, MA) using color values in the red–green–blue (RGB) system. Canopeo analyzes and classifies all pixels in the image. The analysis is based on the selection of pixels according to ratios of R/G, B/G (Liang et al., 2012, Paruelo et al., 2000), and the excess green index (Chen et al., 2010; Richardson et al., 2007). The result of the analysis is a binary image where white pixels correspond to the pixels that satisfied the selection criteria (green canopy) and black pixels correspond to the pixels that did not meet the selection criteria (not green canopy). Fractional green canopy cover ranges from 0 (no green canopy cover) to 1 (100% green canopy cover). The classification of green canopy is based on the following criteria:

$$R/G < P_1 \text{ and } B/G < P_2 \text{ and } 2G–R–B > P_3$$

where P_1 and P_2 are parameters that typically have a value near 1 (Paruelo et al., 2000) to classify pixels that are predominantly in the green band (~500–570 nm), and P_3 is a parameter that sets the minimum excess green index, which typically has a value around 20 to select green vegetation (Meyer and Neto, 2008; Richardson et al., 2007). The default parameter values for Canopeo are $P_1 = 0.95$, $P_2 = 0.95$, and $P_3 = 20$.

Canopeo allows the user to preview the effectiveness of the settings before starting image analysis, which is especially helpful when analyzing a large set of images or videos. Having the opportunity to set, test, and modify threshold R/G and B/G values for several test images selected from the set of images to be analyzed gives the user more confidence in the chosen threshold values. The threshold value for the excess green index was set constant at a value of 20, and cannot be changed by the user. The excess green index effectively classifies dark or gray pixels that cannot be adequately discriminated using the R/G and B/G ratios alone. Canopeo also has the capability to reduce noise by removing isolated green pixels. Isolated pixels that meet the color ratio specifications can sometimes occur in other objects and are not exclusively part of green canopy (Lati et al., 2011). For instance, some isolated pixels in residue shadows may have R/G and B/G ratios similar to those found in green canopies. Canopeo can remove these pixels or small clusters of pixels (e.g., small weeds in a row crop) by analyzing connected neighboring pixels. The user-adjustable noise reduction value in Canopeo determines the minimum number of four-connected pixels that any area in the binary (i.e., classified) image must have to avoid being deleted.

In Canopeo a subset of frames from a given video can be extracted according to user specifications, and then each frame is analyzed as a separate image. Canopeo saves a spreadsheet (Microsoft Excel format) file with the settings used in the image analysis (R/G threshold, B/G threshold, noise reduction), directory, image file name, and FGCC value for each image. In the case of video, Canopeo saves the video file name, frame number, FGCC values, average, standard deviation, coefficient of variation of FGCC in the video, and the minimum number of images that were necessary to estimate the mean with a 95% confidence interval of 0.05 FGCC for each video. The supported video formats are .avi (Audio Video Interleaved format), .wmv (Windows Video Media format), .mp4 (MPEG-4 format), and .mov (QuickTime multimedia file format). Canopeo is available as a free Matlab app, but requires prior installation of a properly licensed copy of Matlab R2013a or later and Matlab image processing toolbox 8.2 or newer. The Canopeo Matlab app can be downloaded by following the link at www.canopeoapp.com. To install Canopeo, first launch Matlab, then open the Apps tab, and finally install the downloaded app. Versions of Canopeo for iOS and Android mobile devices are also available through links at that same website.

Evaluation

Images for the evaluation were typically taken from 1000 to 1400 h on sunny to partially cloudy days during the years 2009 to 2012 from experimental plots in Oklahoma. Nadir (i.e., downward-facing) images were taken from random areas of experimental plots using inexpensive "point and shoot" type digital cameras such as the Canon Powershot SD1200 IS (10 MPX). The camera was kept at about 1.5 m from the top of the canopy using a 1.5 m monopod. Maintaining adequate distance from the camera to the top of the canopy is critical to minimize overestimation of FGCC caused by the top leaves of the canopy being too close to the lens of the camera and by crop rows that are not orthogonal with the lens of the camera (Hoyos-Villegas et al., 2014).

Canopeo was compared against SamplePoint (i.e., MPC) version 1.56 and SigmaScan Pro 5 (i.e., ACT) to test accuracy and image processing speed. The pixel-level accuracy of Canopeo was evaluated by using SamplePoint as the "gold standard" (i.e., best available benchmark). For this accuracy test, we used a set of 20 images with resolution of 8 MPX (3264 by 2448 pixels) and with FGCC ranging from 0.07 to 0.89 (based on values obtained using SamplePoint) for different crops (i.e., wheat, soybean, corn, canola, and grain sorghum) and backgrounds (i.e., no-till and conventional till). For each test image, each pixel in an automatically-created, uniformly-spaced 10 by 10 grid (i.e., 100 pixels) was manually classified as "Green" or "Not-Green". SamplePoint automatically selects the grid spacing, based on the image resolution, so that the grid spans the majority of the image. A total of 2000 manually classified pixels (100 pixels per image × 20 images) resulted in a wide gamut of colors for testing Canopeo's classification accuracy.

The 20 images were classified in SamplePoint by three trained users to account for different perceptions of green canopy cover. Because only two classification outcomes were possible (i.e., Green and Not Green), the final pixel classification was decided on the decision of two out of the three users (i.e., decision of the majority). Pixel classification by the trained users was based on two criteria: (i) color of the central pixel selected by SamplePoint and (ii) surrounding context of the selected pixel. Examination of the context around the pixel (i.e., zooming out from pixel to leaf or plant level) can sometimes reveal that the selected pixel represents green canopy cover, particularly in portions of the image that display shaded canopy. Pixels were classified as "Green" only if the surrounding context of the pixel allowed the user to unambiguously determine that the pixel was representing green canopy cover, otherwise the pixel was classified as "Not-Green".

The same set of 20 images was also analyzed with Canopeo. We developed a Matlab script that matched the pixels selected by SamplePoint with the corresponding pixels classified by Canopeo. Because our pixel classification had a binomial outcome (i.e., Green and Not-Green), we evaluated Canopeo's accuracy using the concept of sensitivity and specificity. Sensitivity evaluates the proportion of true positive cases (i.e., Green pixels defined by SamplePoint), while specificity evaluates the proportion of true negative cases (i.e., Not-Green pixels defined by SamplePoint) cases. Additionally, 50 or more images for corn, forage sorghum, switchgrass, and bermuda grass collected under natural lighting conditions in various fields were analyzed to extend our comparisons of the FGCC values from the three software packages across diverse vegetation, soil, and lighting conditions. We used the RMSD to describe the performance of Canopeo relative to SamplePoint and SigmaScan.

The Canopeo speed test was performed against SigmaScan using a computer with an Intel Core duo2 processor with a speed of 2.66 GHz and 3 GB of RAM. Three sets of 72 images per set were used to evaluate the processing speed using the macro for batch image analysis developed by Karcher and Richardson (2005). The processing speed was measured dividing the total image processing time by the total number of images in the set (i.e., 72 images). For SigmaScan we used a digital stopwatch to measure the processing time of the 72 images, while for Canopeo we used Matlab's stopwatch timer functions (i.e., tic and toc functions). The three sets of images had resolutions of 0.3 MPX (640 by 480 pixels), 3.1 MPX (2048 by 1536 pixels), and 8 MPX (3264 by 2448 pixels), with approximate image sizes of 150 KB, 1.7 MB, and 3.5 MB, respectively. The approximate pixel size for each image resolution assuming a field of view of 1.2 m^2 at the top of the canopy was 4, 0.4, and 0.15 mm^2, respectively.

For SigmaScan, we employed threshold values similar to those used by Purcell (2000), with hue values ranging from 25 to 150 and saturation values ranging from 10 to 115 (Table 1). The hue and saturation values for SigmaScan were optimized based on visual inspection of the classification performance of three to five images within each image set. The threshold values for the R/G and B/G ratios in Canopeo were optimized in the same way, but varied over a smaller range and required less adjustment. The R/G and B/G threshold ratios are independent of each other, and for FGCC the optimal values for both ratios typically ranged between 0.9 and 1. The noise reduction parameter in Canopeo was set to one (effectively disabling the function) to provide a more fair comparison against the other software packages (Table 1).

To demonstrate the spatial variability of FGCC, and how this spatial variability could be assessed by using video recordings, a power analysis statistical procedure was used to calculate the minimum number of images required to obtain a 95% confidence interval of ±0.05 FGCC about the population mean along a 40-m transect in a wheat field (crop stage Feekes 3.0) and a grain sorghum field (crop stage V10). The population mean was calculated as the average FGCC of all images in a

Table 1. Software settings used to analyze each batch of images.

Software	Settings	Corn	Forage sorghum	Turf	Switchgrass
Canopeo	R/G†	0.97	0.97	0.99	1.1
	B/G	0.97	0.97	0.99	1.1
	Noise reduction	1	1	1	1
SigmaScan	Hue range	40–140	40–140	40–140	50–180
	Saturation range	15–100	15–100	15–100	10–100
SamplePoint	Number of pixels	100	100	100	100

† R/G, red to green; B/G, blue to green.

video. The minimum number of samples was calculated using the following equation (Clewer and Scarisbrick, 2001):

$$n=\left(\frac{1.96\sigma}{\delta}\right)^2$$

where n is the number of samples needed to estimate the population mean within $\delta = 0.05$ FGCC with 95% confidence using standard deviation σ, which is calculated from all images in a given video. This comes from the fact that $\bar{x} \pm 1.96(\sigma/\sqrt{n})$ defines a sample mean with 95% confidence interval assuming a known σ. The value of $\delta = 0.05$ FGCC is an arbitrary but reasonable confidence interval width for our purposes.

RESULTS AND DISCUSSION

The combination of the R/G and B/G ratios with the excess green index resulted in effective and rapid classification of FGCC from digital images. Live green vegetation was effectively separated from the background by using the R/G and B/G ratios, which have been proven useful to quantify aboveground live biomass in a perennial shortgrass steppe in north-central Colorado (Paruelo et al., 2000). The excess green index was used to set a minimum pixel greenness in order for a given pixel to be considered green canopy. The excess green index was particularly effective for classifying green canopy cover under dark conditions. The addition of the excess green index avoided mis-classifying dark pixels that otherwise would have been selected by solely using the R/G and B/G ratios. The excess green index can be seen as a variable similar to that of saturation in the hue-saturation-brightness color framework. The excess green index has previously proven useful to track canopy green-up in a deciduous broadleaf forest located in northeastern United States (Richardson et al., 2007).

The classification process using the R/G and B/G ratios is illustrated using an image of no-till canola (Fig. 1A). This image was processed with R/G and B/G thresholds set to 0.95, the excess green index threshold set to 20, and the noise reduction parameter set to 1.0, resulting in FGCC = 0.54. Varying the R/G and B/G thresholds independently between 0.9 and 1.0 resulted in FGCC values from 0.52 to 0.56, highlighting the narrow variability in FGCC when adjusting the R/G and B/G thresholds within this range. The bivariate histogram (Fig. 1B) for this image revealed the formation of two clusters, which represent the green vegetation and the background. The cluster located on the left side of Fig. 1B (Cluster 1) consists of pixels that were classified as Green, while the pixels in the cluster on the right side of Fig. 1B (Cluster 2) consists of pixels belonging to the background (e.g., soil, crop residue, etc.). The clusters in Fig. 1B are mainly a consequence of the bimodal distribution of the R/G ratio (Fig. 1C), which allows for selecting a clear FGCC classification threshold (R/G = 0.95). Nonetheless, the inclusion of the B/G ratio (Fig. 1D) increases the robustness of the classification so that it can be used in more diverse field scenarios such as analyzing zenith (i.e., upward-facing) images where the color of the sky may need to be filtered (Fuentes et al., 2014).

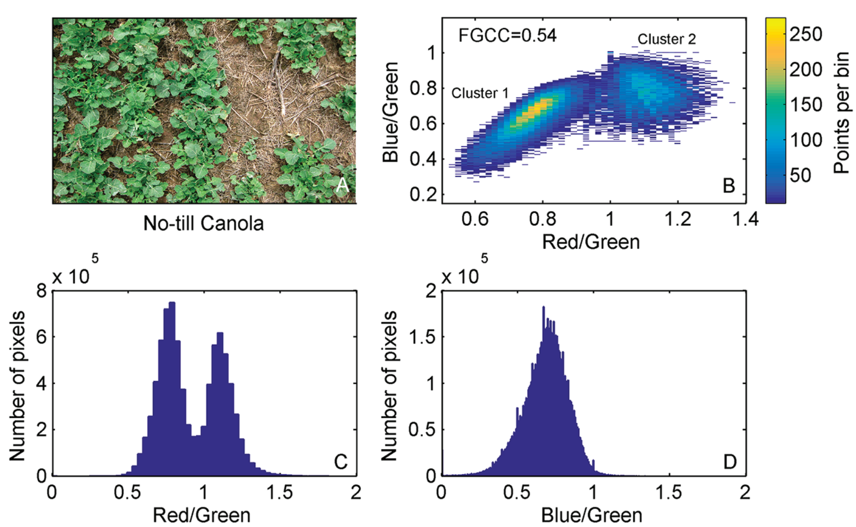

Fig. 1. Histograms of the red/green (R/G) and blue/green (B/G) ratios used to classify fractional green canopy cover (FGCC). Example is presented for (A) an image of no-till canola using (B) a bivariate histogram and the one-dimensional (C) R/G and (D) B/G histograms.

The accuracy test revealed that Canopeo had a sensitivity of 864/(864+89) = 0.91 and specificity of 933/(933+114) = 0.89 (Table 2). These values mean that Canopeo correctly classified 91% of the pixels defined as Green (i.e., true positives) and 89% of the pixels defined as Not Green (i.e., true negatives) by SamplePoint. The fact that the specificity was 2% lower than the sensitivity shows that Canopeo had a slight tendency to classify pixels as Green even though the trained users classified them as Not Green (i.e., false positive, type I error). Overall, Canopeo accurately classified 90% of the pixels (i.e., [864+933]/2000). Manual pixel classification with SamplePoint was used as the pixel-level "gold standard" for comparison in this study, but subjectivity in classification cannot be completely eliminated. At the pixel level, there is sometimes no clear distinction of green canopy cover from background due to pixelation, poor lighting, or other complicating factors.

Canopeo was 20 to 130 times faster than SigmaScan and 75 to 2500 times faster than SamplePoint (Table 3). Image resolution more severely impacted the image processing speed of SigmaScan than that of Canopeo. At the lowest resolution (640 by 480 pixels), Canopeo required 0.12 s per image, and at the highest resolution (3264 by 2448 pixels), 1.6 s per image. On the other hand, SigmaScan required 2.4 s per image at the lowest image resolution and 49 s per image at the highest resolution. The relatively low processing speed of SigmaScan may justify the use of video graphics array (VGA) image resolution, that is, 640 by 480 pixels, when analyzing FGCC with SigmaScan (Purcell, 2000). In SamplePoint, image processing time ranged from 2 to 5 min. Because SamplePoint requires pixel classification by a trained user, the processing speed highly depends on the ability of the user to keep focus on the analysis. The high image processing speed achieved by Canopeo is a result of Matlab's efficiency in matrix manipulation.

The use of Matlab for agronomic image analysis has been reported in other studies. Lati et al. (2011) provided a thorough analysis of the performance of an image segmentation approach based on a hue-invariant transformation to quantify weed biomass and leaf-cover area. The strength of the hue-invariant segmentation approach is the excellent performance under different illumination conditions and camera positions. However, this approach requires a reference point, and was only tested on Purple nutsedge (*Cyperus rotundus* L.) under simple background conditions (i.e., conventional tillage). On the other hand, a study by Robson et al. (2013) used red and green/blue adjustable thresholds to quantify canopy establishment in biofuel crops such as *Miscanthus* (Robson et al., 2013). However, the studies by both Lati et al. (2011) and Robson et al. (2013) were based on single plant species and do not provide detailed information about accuracy and processing time relative to commercially available software packages.

For several crops, Canopeo resulted in FGCC values similar to those obtained using SamplePoint and SigmaScan (Fig. 2). Root mean squared differences for the FGCC values between Canopeo and SamplePoint ranged from 0.056 to 0.123 with an average of 0.086 (Fig. 2). SamplePoint whole-image FGCC estimates cannot be considered as a "gold standard" in this context (i.e., Fig. 2) because the resulting FGCC in SamplePoint was obtained from the analysis of only a small fraction of the pixels in each image (i.e., 100 pixels) whereas the FGCC in Canopeo was calculated by classifying all the pixels. Therefore, the RMSD values shown for the comparisons between Canopeo and SamplePoint result from errors in both approaches. The comparison between SigmaScan and SamplePoint (data not shown) resulted in RMSD of 0.051 for corn, 0.066 for sorghum, 0.096 for turf, and 0.115 for switchgrass, values that are similar to those observed between Canopeo and SamplePoint. The RMSD between FGCC values for Canopeo and SigmaScan ranged

Table 2. Comparison of pixel-level classification by Canopeo and SamplePoint using a total of 2000 pixels selected from 20 images with different crops, backgrounds, and light conditions.

Reference condition	Canopeo Green	Canopeo Not Green	Percent correct
			%
SamplePoint Green	864 (true positives)	89 (type II error)	0.91†
SamplePoint Not Green	114 (type I error)	933 (true negatives)	0.89‡
Correctly classified pixels			0.90

† Sensitivity.

‡ Specificity.

Table 3. Comparison of pixel classification method, processing speed, cost, number of pixels included in the image analysis, and flexibility of Canopeo, SigmaScan, and SamplePoint.

Characteristics	Canopeo	SamplePoint	SigmaScan
Pixel classification	computer	manual	computer
Speed† (seconds per image)			
640 × 480 pixels	0.12	120–300‡	2.4
2048 × 1536 pixels	0.18	120–300	24
3264 × 2448 pixels	1.62	120–300	49.2
Cost (US$)	free§	free	US$999#
Number of pixels analyzed	all	50–225	all
Ability to classify other than green	limited	highly flexible	limited

† Tested with a set of 72 images per level of resolution.

‡ Maximum of 50 to 70 images per day per user.

§ Matlab software needs to be previously installed. Individual academic license was $500 and image processing toolbox was $200 in September, 2014.

Academic price. Commercial price was $1499 on August 2014.

from 0.04 to 0.076 with an average of 0.050. These RMSD values are lower than those obtained by comparing either of the ACT methods to SamplePoint, likely due to the fact that both Canopeo and SigmaScan classify every pixel in the image.

Corn and sorghum images with FGCC near a value of one had a combination of both green leaves fully exposed to the sun light and shaded leaves close to ground level. Despite the challenge of accurately identifying leaves under these difficult lighting conditions, Canopeo detected all green parts of plants exposed to sun light, and a great portion of shaded leaves. For example, see the circled portions of Fig. 3C and 3D. Some shaded lower leaves of the corn canopy are barely visible in the raw image (Fig. 3D) but are accurately identified by Canopeo (Fig. 3C). The Canopeo classification approach demonstrated robust performance even for imperfect images such as when the user's feet or the camera monopod's shadow were present in the images (e.g., Sharma and Ritchie, 2015). Previously, the G/R ratio was used by Adamsen et al. (1999) to measure senesced leaves in wheat (*Triticum aestivum* L.) and by Ritchie et al. (2010) and Sharma and Ritchie (2015) to measure FGCC in cotton. Also, a greenness index using the G/B ratio was developed by Crimmins and Crimmins (2008) to monitor plant phenology. The use of the R/G and B/G ratios together with the excess green index has not been widely reported, but it seems to be highly effective for selecting FGCC of numerous plant species across diverse backgrounds and light conditions without the need for a reference board (Lati et al., 2011).

SigmaScan uses the hue-saturation-brightness (HSB) system instead of the RGB system because the red and blue levels alter pixel greenness (Ewing and Horton, 1999). Many ACT approaches are based on the selection of saturation and hue threshold values, but finding the optimal thresholds is difficult, and threshold settings differ when using images taken under different light intensities and backgrounds (Karcher and Richardson, 2005). Furthermore, in SigmaScan the user cannot pre-visualize the effects of the selected hue and saturation values before running an entire batch of images.

For the batch of turf images, Canopeo had excellent agreement with SigmaScan (Fig. 2, RMSD = 0.041), showing the potential of Canopeo to be applied in turf research and management. On the other hand, greater discrepancies were observed when comparing SamplePoint and Canopeo for turf (Fig. 2, RMSD = 0.092). The grid size of 100 points used in SamplePoint may be inadequate for precise FGCC estimation for images containing turf plugs growing radially from the center of the image (Fig. 4A). Switchgrass FGCC measurement presented a challenge to all three programs because green parts of the plant were mixed with senesced leaves creating a wide range of color tones (Fig. 4B). In fact, close inspection at the pixel level revealed that gray–green and gray–reddish pixels (e.g., R = 90 G = 80 B = 92) were common in actively growing switchgrass leaves, requiring R/G and B/G ratios to be slightly larger than 1 (i.e., R/G = 1.1 and B/G = 1.1, Table 1) to select

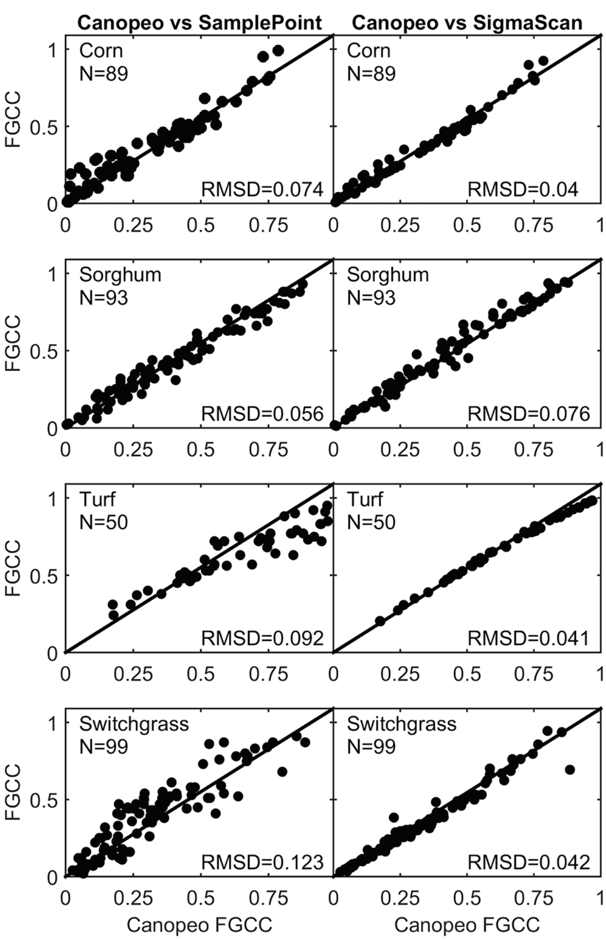

Fig. 2. Comparison of fractional green canopy cover (FGCC) for corn, forage sorghum, turf, and switchgrass using Canopeo, SigmaScan, and SamplePoint. The solid line in each subplot represents the 1:1 line.

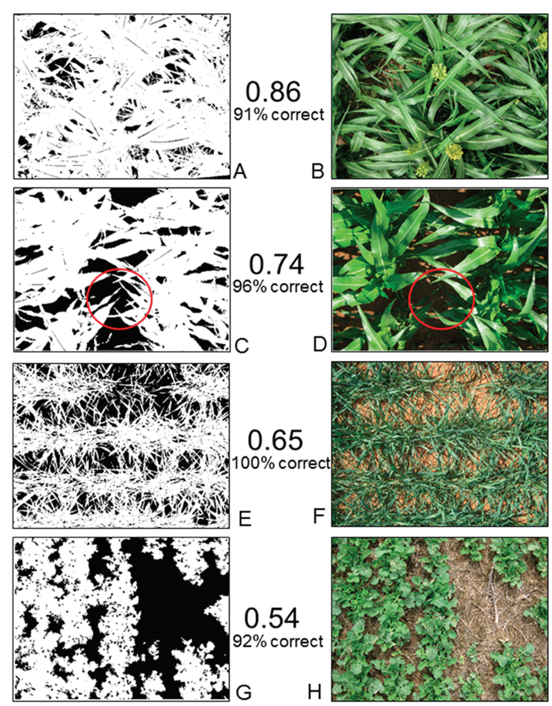

Fig. 3. From top to bottom, digital images of (A, B) no-till grain sorghum , (C, D) no-till corn, (E, F) conventional till wheat, and (G, H) no-till canola are shown after the digital image was analyzed (left) relative to the original image (right). Area in white represents green pixels selected by Canopeo. The fractional green canopy cover from Canopeo and the percent of correctly classified pixels relative to SamplePoint are shown between the images. Area within red circle shows lower leaves in the canopy.

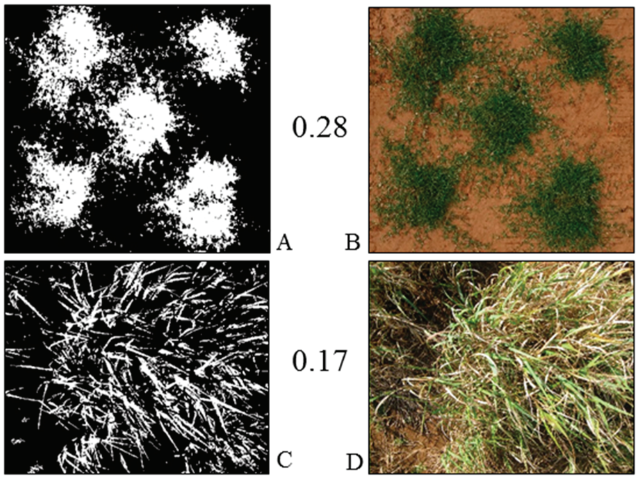

Fig. 4. From top to bottom, digital images of turf (A, B) and switchgrass (C, D) are shown after the digital image was analyzed (left) relative to the original image (right). The fractional green canopy cover from Canopeo is shown between the images.

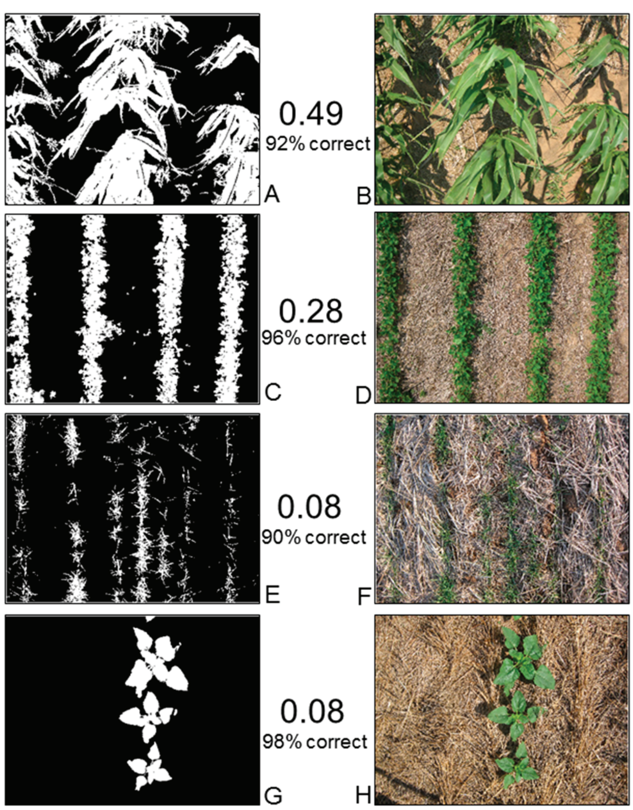

Fig. 5. From top to bottom, digital images of (A, B) no-till grain sorghum, (C, D) no-till soybean, (E, F) no-till wheat, and (G,H) no-till sunflower are shown after the digital image was analyzed (left) relative to the original image (right). Area in white represents green pixels selected by Canopeo. The fractional green canopy cover from Canopeo and the percent of correctly classified pixels relative to SamplePoint are shown between the images.

live vegetation. In this case, SamplePoint may be able to provide the most accurate results, but to achieve good precision for these highly heterogeneous images, a larger grid size (i.e., 225 pixels) may be necessary.

The thresholds were easy to set in Canopeo due to its interactive functionality that allows the user to preview and compare with the original image the effect of the selected R/G and B/G threshold values. This interactive capability allows the user to set proper ratios even under difficult scenarios. Often, no-till cropping systems with low FGCC values are difficult to analyze accurately because crop residue does not offer adequate background contrast. However, Canopeo effectively selected FGCC in no-till crops with low FGCC and complex backgrounds with high crop residue levels (Fig. 5).

Canopeo, as any other measurement tool, relies on proper operation by the end user, and it cannot compensate for some user operational errors. As an example, the images in Fig. 5C and 5G would result in different FGCC if the camera lens had been positioned at different heights from the top of the canopy. In Fig. 5C, the soybean rows on both sides of the image may have been excluded if the camera lens were closer to the canopy. On the other hand, in Fig. 5G additional sunflower rows would have been included in the image if the camera lens was positioned at a greater height above the top of the canopy. While the position of the camera lens can affect the portion of the crop being captured in the image, the classification accuracy of Canopeo remains unaltered.

Perhaps the most unique capability of Canopeo compared to current software packages for quantifying FGCC is the possibility to analyze video recordings. Analyzing video recordings to quantify FGCC can help minimize sampling error in plots or fields with high FGCC spatial variability by allowing the user to record a large number of images in a small amount of time. Video recordings have been used by other researchers to study the severity of foliar plant disease by monitoring necrotic and intact leaves in real-time (Lindow and Webb, 1983). Video was also used to analyze real-time Lepidoptera defoliation in traditional and *Bacillus thuringiensis* (Bt) transgenic cotton in the laboratory, allowing the inclusion of feeding activities in the analysis (Alchanatis et al., 2000). A Matlab tool was used by Fuentes et al. (2014) to estimate LAI in grapevine canopies by recording zenith (i.e., upward-facing) videos. Using zenith images and videos to estimate LAI or FGCC ensures good contrast between live vegetation and its background, which facilitates pixel classification, but operational data acquisition in agricultural fields can be challenging. Shrestha and Steward (2003) developed a vehicle-mounted video system to quantify plant population in Iowa corn fields at a speed of 1 to 2 m/s. That approach was later used by Thorp et al. (2008) to relate airborne hyperspectral remote sensing to ground machine-vision measurements of plant population of corn. In this context, measurement of FGCC from videos has the potential to be integrated with other data streams (e.g., multi-spectral or hyperspectral reflectance, plant height sensing) for high throughput phenotyping of plants.

Recording videos in the field did not take longer than taking still images. About 30 s were required per plot to record a 15-s video, covering an approximate area of 15 m^2. Fifteen seconds of video using a Canon Powershot SD1200 IS (10 MPX) camera at 30 frames per second resulted in 450 frames. Even though each frame had VGA resolution, each frame is equivalent to an image.

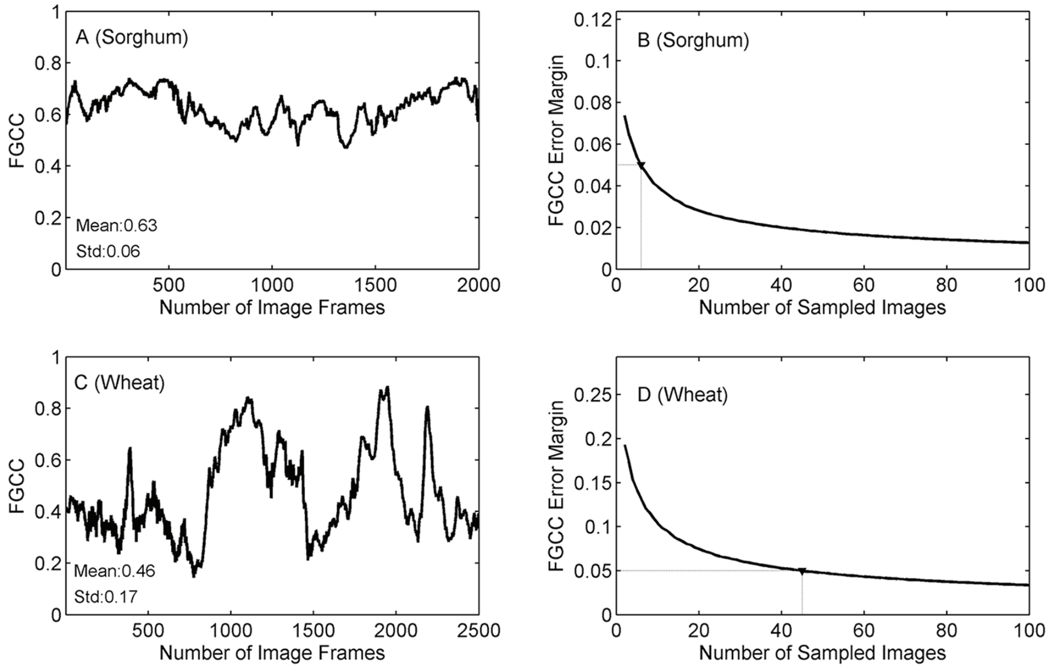

Fig. 6. Fractional green canopy cover (FGCC) showing the variability along a transect in a (A) grain sorghum field at phenological stage V10 and (C) a wheat field in stage Feekes 3.0. The minimum number of images required to have a 95% confidence interval of ±0.05 about the mean FGCC is shown in B and D.

Also, many digital cameras allow for high definition video recordings. In the presence of spatial variability of FGCC, videos allow the FGCC of a field or experimental plot to be estimated more accurately by obtaining values close to the FGCC population mean rather than estimating FGCC based on a few images. This advantage is especially significant in plots or fields where FGCC shows large spatial variability and many images may be required to obtain a representative mean.

An example FGCC transect of ~40 m length in a grain sorghum field in growth stage V10 is presented in Fig. 6A. The mean FGCC of the transect using ~2000 images was 0.63 with a standard deviation of 0.06 and a coefficient of variation of 9.5%. Using power analysis, we determined that six images were required to have a 95% confidence interval of ± 0.05 FGCC about the mean (Fig. 6B). On the other hand, a transect of ~40 m in a wheat field in growth stage Feekes 3.0 had a mean of 0.46 FGCC and substantial spatial variability across the recorded transect with a standard deviation of 0.17 and coefficient of variation of 37% (Fig. 6C). The minimum number of images needed to have a 95% confidence interval of ±0.05 FGCC about the mean was 45 (Fig. 6D). This shows the importance of large sample sizes in estimating FGCC for heterogeneous canopies and how the use of transect video recordings can help minimize sampling error. Also, the possibility of analyzing FGCC from videos is useful for overcoming the need to choose "representative" locations in heterogeneous canopies and, therefore, minimizing researcher bias.

CONCLUSIONS

Canopeo is capable of detecting FGCC at high speed relative to the available software packages tested in this study without sacrificing accuracy. The video feature present in this tool is a novel addition to software packages that are used to measure FGCC, allowing the user to record a large number of images and therefore minimize sampling error. One limitation of Canopeo (and other FGCC methods based on digital images) is the need to keep the camera an adequate height above the canopy. For vegetation taller than about 2.5 m, this may require the use of aerial images or special equipment. It may be possible to use the R/G and B/G ratios together with the excess green index to detect other components of digital images, but this possibility requires further research. It is important to highlight that MPC programs such as SamplePoint are invaluable for calibration and when there is need for simultaneous estimation of more complex variables other than FGCC. The Canopeo app for Matlab, as well as versions for iOS and Android mobile devices, can be downloaded at www.canopeoapp.com. The mobile apps are powerful tools which allow producers, crop consultants, researchers, and other end users to easily acquire, process, and annotate digital images in the field to obtain real-time, geo-referenced green canopy cover estimates. The images, FGCC estimates, and associated metadata can be sent to a designated web server for future access and reporting.

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