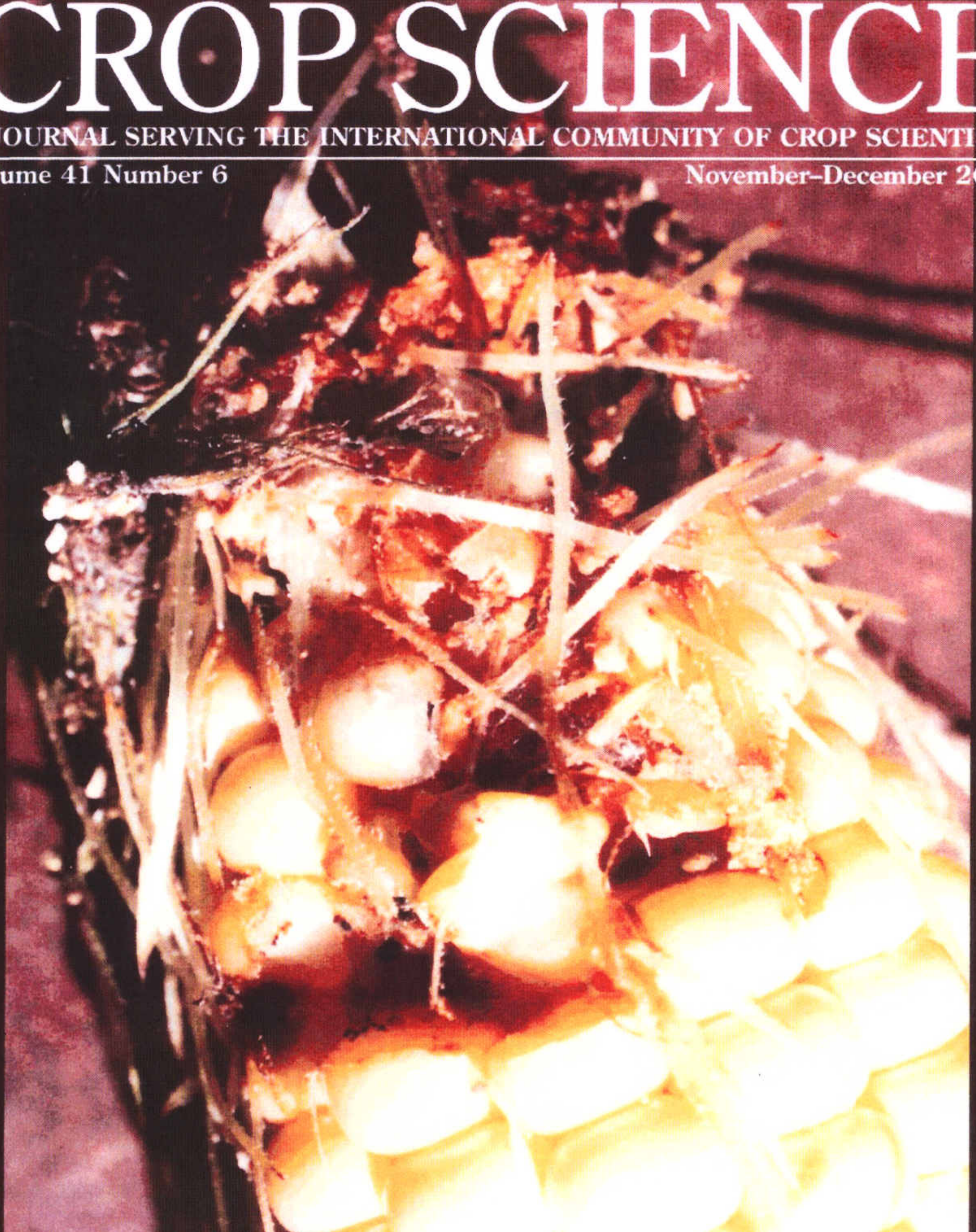
CROP SCIENCE

A JOURNAL SERVING THE INTERNATIONAL COMMUNITY OF CROP SCIENTISTS

Volume 41 Number 6 November–December 2001



CRPSAY 41(6) 1657–2042 (2001)

Quantifying Turfgrass Cover Using Digital Image Analysis

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ABSTRACT

Accurate cover estimates in turfgrass research plots are often difficult to obtain because of the time involved with traditional sampling and evaluation techniques. Subjective ratings are commonly used to estimate turfgrass cover, but the data can be quite variable and difficult to reproduce. New technologies and software related to digital image analysis (DIA) may provide an alternative method to measure turfgrass parameters more accurately and efficiently than current techniques. A series of studies was conducted to determine the applicability of DIA for turfgrass cover estimates. In the first study, plots containing a range (1–16) of bermudagrass [*Cynodon dactylon* (L.) Pers.] plugs of specific diameter (15.0 cm) were established to represent values of turfgrass cover from 0.75 to 12%, by 0.75% increments. Digital images (1280 by 960 pixels) were taken with a digital camera and processed for percent green color to a software package. Estimates of green turfgrass cover by DIA were highly correlated ($r^2 > 0.99$) to the calculated values of turfgrass cover. In a second study, DIA of turfgrass cover was compared by subjective analysis (SA) and line-intersect analysis (LIA) methods for estimating cover in eight plots of zoysiagrass (*Zoysia japonica* Steudel). The mean variance of percent cover determined by DIA (0.65) was significantly lower than SA (99.12) or LIA (13.18). Digital image analysis proved to be an effective means of determining turfgrass cover, producing both accurate and reproducible data. In addition, the technique effectively removes the inherent error and evaluator bias commonly associated with subjective ratings.

UNDERSTANDING factors that influence establishment is an important aspect for any agricultural crop. This is especially true for turfgrasses, where improper or delayed establishment can cause significant problems that may persist for the life of that crop. Unfortunately, turfgrass establishment studies are often challenging under field conditions because of the time and labor involved in measuring and quantifying development and establishment parameters. For most establishment studies, data that are collected will include either a small number of sample dates where accurate, quantitative data are collected, or frequent, subjective evaluations, where visual ratings are used to estimate growth (Skogley and Sawyer, 1992). Although quantitative data are generally more precise than subjective data, the time and costs associated with collection of quantitative information often limits its use.

Most quantitative analyses of turfgrass establishment and cover include subsampling from a plot followed by a nondestructive or destructive analysis of plant populations. Nondestructive analysis of fixed or random quadrats can be effectively used to determine plant density or cover in grass crops, but the data can be highly variable and subtle differences due to treatments are difficult to identify as a result of the high variances (Murphy et al., 1995). The line-intersect method is commonly used for ecological studies in which the occurrence of plants or the distribution of plant types within a plot are required (Laycock and Canaway, 1980; Kershaw, 1973). The line-intersect method involves setting up a grid system over an entire plot or a quadrat within the plot and counting the number or types of plants found at each intersection on the grid. The number of intersects where the desired plant material is found is then multiplied by the area of each grid section and divided by the total sample area for a percentage of each species. The number and size of grids sampled from each plot or quadrat will be dictated by the accuracy or precision needed and the time and labor necessary to complete the task. The line-intersect method has been effectively used in many forms of ecological research, but the time and labor required for data collection can limit the scope of a study.

The most commonly used technique for estimating turfgrass cover or rate of spread in turfgrass establishment studies involves frequent, subjective ratings by trained evaluators. This type of measurement is used for cultivar evaluation trials and other management studies (Morris, 2000). Although relevant information can be collected from these types of studies, the resultant data can be variable and difficult to reproduce by other investigators. In a study by Horst et al. (1984), 10 trained turfgrass researchers subjectively rated the same turfgrass stands for quality and density to determine the uniformity of their evaluations. In that study, more variation was found to be associated with the individual evaluator rather than the cultivars evaluated. Although these investigators concluded that subjective evaluations of turfgrass plots were inadequate in most situations, these methods continue to be used extensively over 15 yr later.

Multispectral radiometry has also been applied to the evaluation of turfgrass quality parameters (Trenholm et al., 1999). Trenholm and coworkers (1999) collected reflectance data from several established turfs over a range of wavelengths and compared the data with components of turfgrass swards such as turf quality, density, shoot-tissue injury, and growth. Although modest correlations were established between this technique and subjective ratings, little information on reproducibility of the technique was provided.

New technologies involving image analysis of digital photographs have recently appeared in the agronomic literature. These technologies have been applied to the determination of color and fertility differences in *Zea mays* L. (Ewing and Horton, 1999), canopy coverage

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Published in Crop Sci. 41:1884–1888 (2001).

Abbreviations: DIA, digital image analysis; LIA, line-intersect analysis; SA, subjective analysis.

and light interception in *Glycine max* (L.) Merr. (Purcell, 2000), and groat characteristics of *Avena sativa* L. (Doehlert et al., 1999). Some of the work using these technologies has involved the development of specific computer programs to identify a desired wavelength or bandwidth and convert those wavelengths into a quantitative parameter that could be analyzed by standard statistical methods (Ewing and Hortin, 1999). However, Purcell (2000) demonstrated the applicability of commercially available software, Sigma Scan (SPSS, Inc., Chicago, IL) to measure canopy coverage in a soybean field and relate those cover determinations to light interception. On the basis of these advances, we hypothesized that DIA could be effectively used to measure turfgrass cover and that the data collection process would be more reproducible and less labor and time consuming than traditional techniques of quantitative or subjective analysis.

MATERIALS AND METHODS

Digital Image Analysis

Digital images were obtained with an Olympus C3030Z (Olympus Optical Co., London, UK) digital camera mounted on a monopod designed specifically for this study. The monopod was constructed from 10-cm-diam polyvinylchloride tubing and consisted of a vertical stand that was 1.5 m in height and a horizontal arm that was mounted at 90° from vertical and extended 1.0 m away from the vertical axis. This allowed the camera to be positioned such that an image could be obtained from directly above the plot without obstructions from the photographer or any part of the camera stand. An infrared remote control was used to release the camera shutter. The collected images were saved in the JPEG (joint photographic experts group, .jpg) format, with a color depth of 16.7 million colors, and an image size of 1280 by 960 pixels. Camera settings included a shutter speed of 1/400 s, an aperture of F4.0, and a focal length of 32 mm. Photos were taken on full sunlight days between 1400 and 1600 h.

Digital images were downloaded to a personal computer and analyzed individually by SigmaScan Pro (v. 5.0, SPSS, Inc., Chicago, IL 60611). The color threshold feature in the SigmaScan software allows the user to search a digital image for a specific color or a range of color tones. Preliminary work with similar images indicated that a hue range from 57 to 107 and a saturation range from 0 to 100 would selectively identify green leaves in the images. After developing a fingerprint of the green areas of the image, the measurement tools in the software package were used to count the total number of selected green pixels. The number of green pixels in each image was then divided by the total pixel count of the image for a determination of turf coverage percentage in the image.

Calibration Curve

To assess the accuracy of the digital camera in determining the amount of green turfgrass cover in a plot, an experiment was conducted in which calibration plots were established using 'Tifway' bermudagrass plugs (15.0-cm diam) in a clean seed bed. Sixteen plots (1.5 by 1.5 m) were developed for the study, with each plot containing a specific number of plugs, ranging from 1 up to 16 plugs. Placement of plugs within the plots began at the center and were increased in a diamond-shaped grid to the edges of the plots. Plugs were taken from healthy and actively growing areas, with the assumption that the area of each plug had 100% green pixels, as determined by software parameters. Pictures were taken immediately after installation of plugs. Because the amount of cover was known for each plot, on the basis of the area of each plug (πr^2) multiplied by the number of plugs, we were able to test how closely the digital images analyzed by SigmaScan predicted the actual amount of cover. Percent cover estimates from digital images were compared with the actual cover by regression analysis (Proc Reg) using SAS Statistical Software (SAS Inc., Cary, NC).

Comparison of Different Rating Techniques

An ongoing field study involving the establishment of zoysiagrass from sprigs was used to determine the applicability of digital image analysis for determining turfgrass cover in comparison with subjective analysis and line-intersect analysis. The study was located at the University of Arkansas Research and Extension Center, Fayetteville, AR (Captina silt loam soil, typic hapludult, pH 6.2). The site was planted with 'Meyer' zoysiagrass sprigs on 4 June 2000. The objective of the experiment was to evaluate different sprig planting methods and various fertility programs during grow-in. Because of the variability in planting and management, a range of cover rates were present in this study.

A subplot (0.91 by 1.21 m) within the existing main plots (2.4 by 2.4 m) was used for all methods of cover estimates. A total of eight plots, with various degrees of cover, were selected for the study. Cover for each plot was determined by three methods: (i) SA ratings by five independent, trained evaluators, (ii) LIA by five trained evaluators, and (iii) DIA on three replicate images as described above. The LIA was performed with the digital images taken for image analysis. Each image was superimposed with a grid to yield square quadrats of 6.0 by 6.0 cm. The same raters were used for both SA and LIA.

The variance for each cover determination method was calculated as an error mean square (removing plot effects) by summing the within subplot squared differences for each method and dividing that sum by the appropriate degrees of freedom (df = the total number of observations for a given method divided by the number of plots). An *F*-test was used to test for significant differences in variance between DIA and the other analysis methods.

RESULTS AND DISCUSSION

Calibration Study

For DIA to be used effectively for turfgrass cover estimates, it was essential to compare percent cover estimates from images with known values of turf cover. The turfgrass plugs that were used to develop the calibration plots represented percent turfgrass covers ranging from 0.75 to 12%, by 0.75% increments. Analysis of the digital images using Sigma Scan clearly identified the green leaves of the turfgrass plugs against the soil background (Fig. 1A). The processed digital images closely predicted the actual percent cover of the calibration plots (Fig. 2), as regression analysis indicated a high correlation ($r^2 = 0.99$) between predicted and actual values, with a slope of 1.01 and an intercept near 0. The coefficient of determination, slope, and zero intercept indicate an excellent fit to the data and a close 1:1 relationship between the predicted and actual percent cover.

The calibration data demonstrate that DIA can accu-

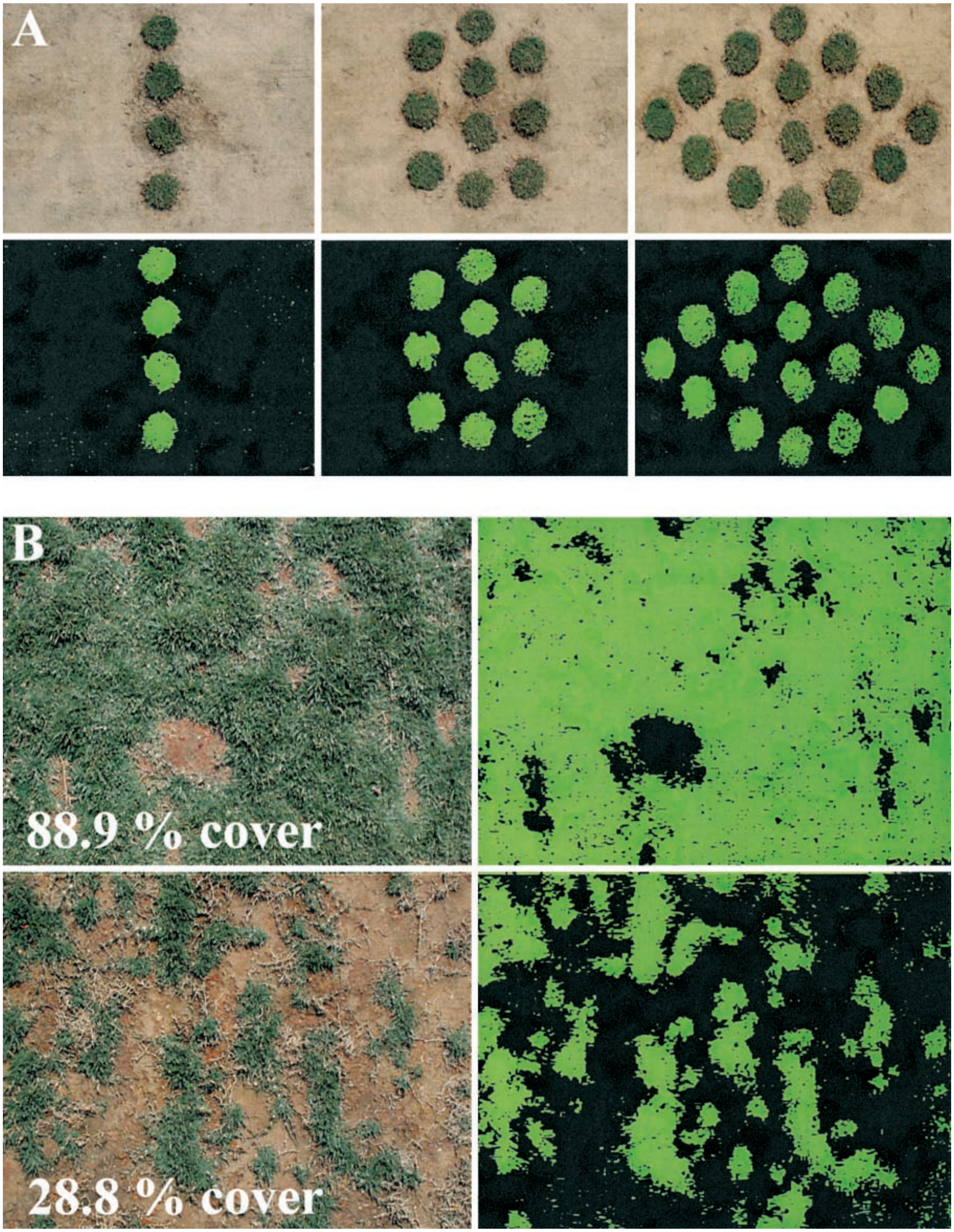

Fig. 1. Digital image analysis of calibration plots (A) and cover estimates (B). In each case, the scanned area of the digital image is displayed concurrently with the original photo. Percent cover estimates in (B) were determined by dividing the number of scanned pixels (green turf) on the right by the total number of pixels in the image.

rately measure the amount of green turf in a chosen turfgrass area. Another quantitative technique that was recently used to assess turfgrass quality parameters involved the use of multispectral radiometry (Trenholm et al., 1999). In that study, visual and near infrared reflectance data collected from a multispectral radiometer were compared with various qualitative and quantitative data in established plots. Trenholm and coworkers (1999) found correlations between radiometer data and subjective ratings ranging from 0.44 to 0.83. Although reflectance technologies are promising for turfgrass quality estimates, the only quantitative data that their tech-

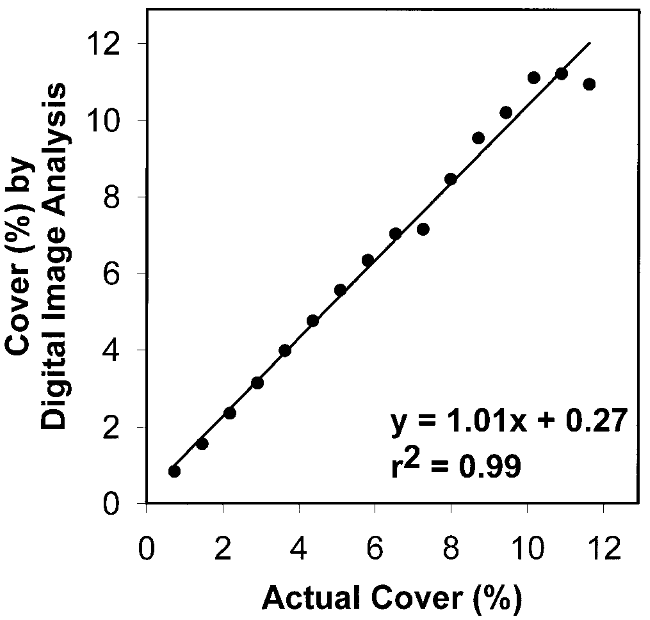

Fig. 2. Regression analysis of actual percent cover values against valued generated by digital image analysis. Variable cover rates were produced from a range of turfgrass plugs of a defined size and area.

Table 1. Comparison of three methods to measure percent turfgrass cover; digital image analysis (DIA), subjective analysis (SA), and line-intersect analysis (LIA).†

Plot	DIA	SA	LIA
	% cover		
1	88.3a‡	81.8a	92.9a
2	69.0d	63.6bc	79.7bc
3	59.6e	53.4c	77.9c
4	83.7b	76.0ab	92.8a
5	74.1c	67.2b	84.3b
6	29.0g	21.0e	50.5e
7	42.8f	39.0d	69.1d
8	58.3e	52.0c	80.2bc
LSD (0.05)	1.40	12.83	4.67
Variance (σ^2)§	0.65	99.12	13.18
F-test comparing DIA vs. LIA and SA¶		153.23	20.28
P-value comparing DIA vs. LIA and SA		<0.0001	<0.0001

† Three digital images, five independent subjective ratings, and five independent line-intersect analyses collected per plot.

‡ Means within a column not followed by the same letter are significantly different at the 0.05 level of probability as determined by LSD.

§ Variance computed for each analysis by removing plot effects; $\sigma^2 = [\Sigma(y_{ij} - y_j)/(n - p)]$
i = 1 to 24, 1 to 40, and 1 to 40 for DIA, LIA, and SA, respectively
j = 1 to 8
n = 24, 40, and 40 for DIA, LIA, and SA, respectively
$p = 8$

¶ F test statistic = $\sigma^2_{SA}/\sigma^2_{DIA}$ and $\sigma^2_{LIA}/\sigma^2_{DIA}$

nique was compared with was shoot growth, as measured by clipping weights. The correlations of reflectance data to shoot growth were generally less than 0.20, suggesting a poor fit of the data in their study. Although other data collected in their study produced higher correlations, the data were being compared with subjective ratings, not quantitative parameters with a known value. Conversely, in the present study, the development of calibration plots with a known amount of turf surface area allowed us to compare the DIA data with a known, quantitative value of turf cover.

Comparison of Different Rating Techniques

Digital images of zoysiagrass plots processed by SigmScan defined turf areas in the digital photos (Fig. 1B). As seen in Fig. 1B, small areas of turf were identified with precision by DIA. All three rating techniques used for the zoysiagrass establishment plots produced a similar ranking of plots from highest to lowest percent turfgrass cover (Table 1). However, there was a significant difference in variation between data collected by SA and DIA ($P < 0.0001$) or LIA and DIA ($P < 0.0001$). The variance for SA was 152 times greater than DIA, while the variance for LIA was 20 times greater than DIA (Table 1). The high variance inherent in LIA and SA may interfere when comparing data across dates or geographic regions. As noted by Horst et al. (1984) in their examination of subjective ratings, more variation was attributed to the evaluators than the actual differences in the plots. By using DIA, individual raters can independently evaluate studies without introducing bias or evaluator variability in the results. In addition, regional or national trial data can be more effectively conducted since the data collected from various sites can be compared in a valid statistical manner.

The computed LSD values for the three evaluation techniques also produced interesting results (Table 1). By means of DIA, seven statistically different groupings were produced from these plots while SA and LIA only separated the data into five groups. The inability to differentiate treatment or cultivar effects is a common problem in many turfgrass trials, especially those that contain a wide range of cultivars or treatments over multiple sites. For example, the National Turfgrass Evaluation Program conducts an array of cultivar trials in which over 100 entries may be assessed at 25 to 30 locations (Morris, 2000). The variances in these subjective ratings are generally so high that separation of performance is only possible between the very high performers and the very low performers, while many of the entries are not significantly different from each other. By implementing techniques such as DIA across multiple sites and evaluators, the variance in data produced will more appropriately reflect location or treatment effects, rather than evaluator effects. It will then be up to the discretion of the researcher to determine which statistical differences are of practical importance.

Because DIA was shown to be accurate in the calibration studies when compared with known values of turfgrass cover (Fig. 2), the data collected by SA and LIA in the zoysiagrass establishment studies were compared with DIA by regression analysis to confirm the accuracy of standard techniques used for turfgrass research studies and assess if these techniques produced bias compared to DIA. Interestingly, subjective estimates were more closely related to DIA ratings in this study than were LIA ratings, as noted by an r^2 of 0.99 for SA compared to an r^2 of 0.93 for LIA (Fig. 3). Also, the SA-produced data followed more closely a 1:1 relationship with DIA, as indicated by a slope of 0.99, across the range of plot cover used in this study. However, the data produced by SA were negatively biased compared

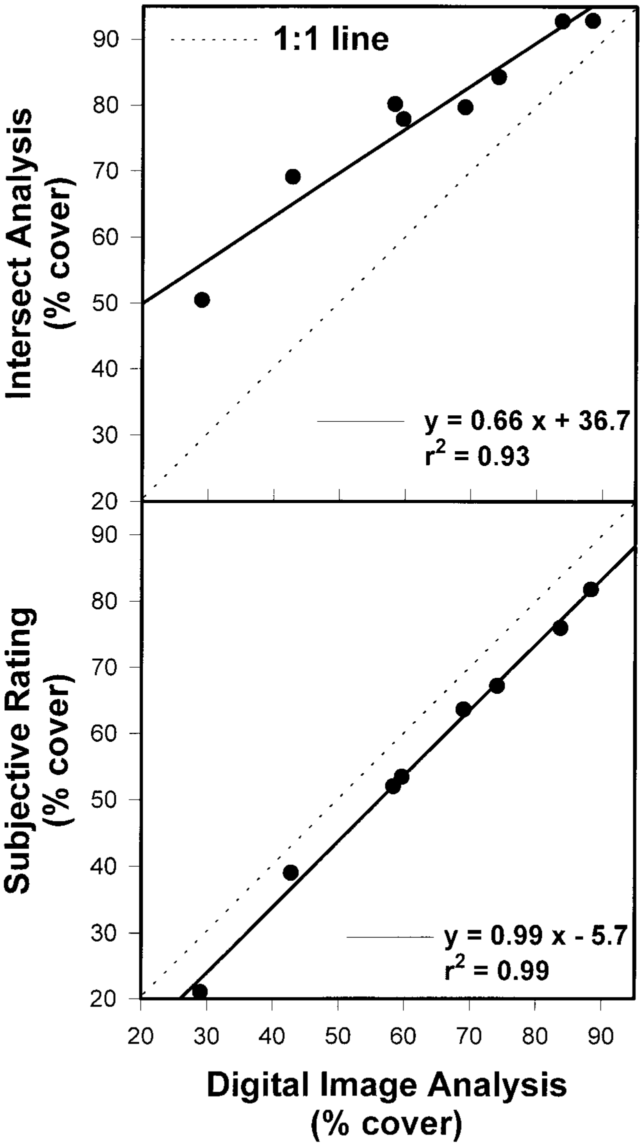

Fig. 3. Regression analysis of percent cover by digital image analysis versus either line-intersect analysis (top) or subjective analysis (bottom). The dotted line on each image represents a 1:1 relationship.

with DIA across the range of turfgrass cover estimates (Fig. 3). Conversely, estimates produced by LIA tended to be positively biased compared with DIA. The estimates of cover by LIA would have been improved had smaller grids with more intersects been used, but this would have added more time to the analysis and further reduced the practicality of LIA for many studies.

SUMMARY

Digital image analysis proved to be an effective method for evaluating the amount of green turf in turfgrass research plots. Estimates made by DIA were more precise than either SA or LIA and were more effective at detecting small, significant differences in cover. In addition, the measurements obtained with DIA were correlated highly to calculated values of turfgrass cover and correlated highly to both SA and LIA estimates.

The accurate measurement of green turf in a plot area has potential in any research study where the amount of green tissue is an indication of health or growth, including turfgrass cover rates of seeded and sprigged grasses, injury ratings of various grasses, and disease or insect injury. Preliminary work (Richardson, 2000, unpublished) suggests that incidence of disease in turfgrass plots can be measured accurately with DIA as the difference between total image size minus the healthy (green) turf area of the image. In addition, SigmaScan may allow pathologists not only to determine the total area of diseased turf, but also to count the number of disease lesions in a field of vision.

In addition to the improved precision and removal of evaluator bias, digital images can be obtained and processed quickly (<2 min per image) and the process can be performed easily by untrained workers. Although the entire process is more time consuming than SA, digital image processing is much more time and labor efficient than LIA, which took approximately 5 to 8 min/plot. This allows the investigator to collect more extensive, quantitative data in these types of studies. Another advantage of DIA is that the development and condition of turfgrass in research plots can be fixed in time by taking a digital image and analyzing it at a later time if necessary.

ACKNOWLEDGMENTS

The authors thank the technical assistance of Gene Bordelon, John McCalla, Margaret Secks, Megan Thomas, and Brandon Wright and financial assistance in the form of a copy of SigmaScan Pro Software from SPSS, Inc.

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