Digitized images provide more accuracy and efficiency to estimate bryophyte cover

JUAN C. BENAVIDES
Department of Biology, University of Puerto Rico, Mayagüez Campus, Mayagüez, PR, 00680; current address: Department of Plant Biology, Southern Illinois University, Carbondale, IL 62901-6509, U.S.A.
email: jbenavid@siu.edu

INES SASTRE-DE JESÚS
Department of Biology, University of Puerto Rico, Mayagüez Campus, Mayagüez, PR, 00680
email: inesdj@caribe.net

ABSTRACT. Bryophytes constitute important components of many ecosystems. However, the methods used to measure their dominance have remained unchanged during the last 25 years. Herein we compare accuracy, efficiency and objectivity among three methods used to estimate bryophyte cover: Braun-Blanquet cover classes, grid percentage and digital image processing. Using these methods, two observers determined bryophyte cover on 30 clay tiles planted with Neckeropsis disticha. Accuracy among methods was estimated by relating the cover values with the dry weight. Efficiency was estimated using the relation between time and data variability. Objectivity was estimated by comparing the variability between observers. The digital method resulted in less time in the field (F = 272, p < 0.001) and lower variation among data (F= 0.55, p = 0.02) than from the other two methods. Our results showed that the digital processing method was more reliable as it minimized observer effect in the cover values while providing higher efficiency in the field. The method is especially useful in monitoring studies that use repeated measurements because it helps to detect small variations in bryophyte cover. This method is particularly useful to improve the analysis in communities with low bryophyte cover, such as tropical epiphylls.

KEYWORDS. Braun-Blanquet, bryophyte cover, digital image analysis, ecological methods, GIS, grid method, objectivity, Neckeropsis.

Despite the importance of bryophyte abundance in ecological processes of tropical ecosystems (Clark et al. 1998; León-Vargas et al. 2006), few studies have explored how to increase precision and accuracy of the methods usually employed to estimate bryophyte dominance. Even though biomass measurements from bryophyte harvesting techniques are acceptable descriptors of bryophyte communities, this method is not practical for monitoring studies due to excessive plant manipulation and loss. Consequently,
the use of methods based on cover are commonly employed, especially the Braun-Blanquet approach, where cover is considered a good descriptor of dominance in non-tree communities (Braun-Blanquet 1932). Many of the techniques employed to determine bryophyte abundance have remained unchanged in the last 25 years and these mainly have consisted of two methods.

The first approach is based on Braun-Blanquet categories (Braun-Blanquet 1932) in which bryophyte species are assigned to discrete cover categories. The cover value is assigned by visual estimation and may include several layers of vegetation. This value scale is divided in a log series in which the first category corresponds to the interval between the species presence and 6.25% of cover and ends in a fifth category that includes the interval between 50–100% of cover (Braun-Blanquet 1932). This method has been widely used with few modifications in phytosociological studies in tropical regions (Duivenvoorden & Cleef 1994; Kessler 2000) as well as in manipulative experiments in temperate regions (Kimmerer 2005). Because this method has low precision, studies that require more accurate cover values have instead used complete quantitative strategies using raw percentages (Holz & Gradstein 2005; Kimmerer 2005).

The second method employs a grid divided into cells of the same size. Here, bryophyte cover is estimated as the percentage of cells occupied by bryophytes. The method has several variations. For example, the measurement may consist of either 1) inclusion of only those cells completely covered by bryophytes, 2) exclusion of those not completely covered and 3) adding incompletely covered cells. This method has been used in studies monitoring germination (Kimmerer 2005) and in bryophyte responses to environmental changes (Uribe & Orrego 2001). The grid percentage method requires more time in the field and it may present a higher variation among observers, due in part to the different methodological approaches.

We consider that any new method for measuring bryophyte cover should be more reliable, efficient and objective than the methods that it intends to replace. For a method to be reliable, the measurements estimated should both accurately describe the natural phenomenon involved and achieve efficiency while minimizing field time and data variation (Krebs 1989). To achieve objectivity, it should aim to minimize the variation among observers (i.e., different observers being indistinguishable from one another).

Herein we demonstrate that using Geographical Information Systems (GIS) software to process bryophyte cover provides a reliable, efficient and objective measure of bryophyte dominance. This approach is useful to describe bryophyte communities because it allows the investigator to study short term dynamics and communities with extremely low cover values.

METHODS

Method description. Although we used the IDRISI Kilimanjaro software (Eastman 2003), the data can, in fact, be processed with almost any GIS-oriented software, such as ERDAS (Anonymous 1994) and Biomapper (Hirzel et al. 2002). The method consists of several steps (Fig. 1): 1—Photograph a bryophyte sample in a demarked area with a digital camera; 2—Import the image to the GIS environment as a Raster image (*.rst), in this step the image will change from 4 MB to more than 17 depending on camera resolution; 3—Perform a manual analysis using digitized polygons equivalent to drawing vegetation types in landscape analysis (in this case, species may be separated by assigning different codes to polygons and thereby representing the different species); and 4—Perform an automatic (unsupervised) bryophyte cover analysis that separates the original image into its three basic colors and then regrouping these colors using, in this case, the cluster module on IDRISI Kilimanjaro (Eastman 2003). However, different classification techniques can be used. For example, the module Cluster selected both the most frequent pixel values in the different bands and the histogram peaks. Thereafter, it assigned the neighborhood pixels to the closest peak. The clusters selected reduce the variation in the three images separated from the original image, reducing the original 256 levels in each of the three images to 4–8 categories. In this case, the Cluster module, set at the predetermined options for all users, was run on all the images. It performed a linear
stretched algorithm with 1% saturation and retained all the clusters found. The cluster image is reclassified as Boolean by assigning values of zero and one to non-bryophyte and bryophyte covered areas, respectively. Finally, the Boolean image was multiplied by the manual classification values (obtained from the third step) to obtain a final cover image using both automatic and manual techniques. The area values are extracted from queries available in the GIS software. A complete description of the digital image technique process in the IDRISI software is available at (http://academic.uprm.edu/~isastre/); the file is available as an IDRISI macro modeler file that runs the manual and automatic classifications of the images such that the final areas are obtained.

**Data Set.** We designed an experiment to verify the reliability, efficiency, and objectivity of the digitized photographs. Thirty clay tiles (20 × 20 cm) were planted with *Neckeropsis disticha* in different arrangements. In order to make them more similar to natural substrates, the samples included the additions of dead leaves, small fragments of wood and debris. Two different observers measured bryophyte cover using three methods: Braun-Blanquet scale, grid percentage and digitized photographs. For the grid percentage method, we used a grid with a mesh size of 2 cm and it covered entirely the 20 × 20 cm tile. We also measured the dry weight of the *N. disticha* arrangements after removing the non-bryophyte objects on the tile to obtain an independent measurement of the dominance. The two observers used the same set of original images and were relatively familiar with, though not experts in, the three methods employed.

**Analysis.** We estimated the reliability of the method using a regression between each cover method and the weight of the sample and compared the resulting correlation values among methods using a Fisher modified test with the package compOverlapCorr in R (Li & Zhu 2006; R Development Core Team 2006). We determined the efficiency with Wiegert’s method by comparing the product between the relative variation and relative time consumed among methods (Krebs 1989). The method with the lowest product was selected as the more efficient and, therefore, most appropriate for the particular study (Krebs 1989). Finally, we compared the objectivity by relating the mean cover and variance between observers in a paired t-test. The variation between observers used a modified Levene test based on the absolute value of the residuals of the linear model (Cover = Observer + Observer (Tile)). We employed a Tukey HSD Post hoc test to identify significant differences between means. We transformed the grid percentage with the arcsen function and dry weight with natural log to fulfill normality requirements (Sokal & Rohlf 1995). All the analyses were done using the R statistical software (R Development Core Team 2006).
RESULTS

The different methods employed to measure bryophyte cover were highly correlated with dry weight (Braun-Blanquet method $r^2 = 0.8$, Grid percentage method $r^2 = 0.81$ and analysis of digital image method $r^2 = 0.86$); that is, there were no differences among the correlation coefficients of cover versus dry weight in the three methods. Digital image cover estimation showed both a higher efficiency with significant lower cost in the field ($F = 272$, df1 = 2, df2 = 87, $p < 0.001$) and lower variation among the data ($F = 0.55$, df1 = 2, df2 = 59, $p = 0.02$) than the other two methods (Fig. 2).

The most efficient method for estimating bryophyte cover was the digital photograph as this showed the lowest data variation and time investment in the field (Table 1). The grid percentage method yielded both higher variation values and invested time, while the Braun-Blanquet showed intermediate values for both of these parameters. The mean cover measured by both observers was similar in the Braun-Blanquet and digital image method, but the grid percentage method showed different means between observers ($t = 6.84$, $p < 0.001$). In the same manner, the variation revealed in the bryophyte cover was different between observers only for the grid method ($t = 5.2$, $p < 0.01$) (Fig. 2). An additional consideration is the time spent in the laboratory. On this point, it is notable that the processing of each digital image required from four to six laboratory minutes, with the Braun-Blanquet and grid percentage methods keeping this time investment to a minimum.

DISCUSSION

Geographical Information Systems have been widely used in bryophyte macroecology mainly to describe landscape patterns (Vanderpoorten et al. 2005; Zechmeister et al. 2002). Moreover, it has been employed beyond its original intention by being applied in small scale studies (Vanha-Majamaa et al. 2000). The method herein proposed to estimate bryophyte cover has several advantages over the traditional methods: it is faster in the field, yields lower variance and the differences among measurements made by observers is lower than in the other methods considered. Despite this, the digital

<table>
<thead>
<tr>
<th>Method</th>
<th>Variation (SD)</th>
<th>Standardized variation (Sv)</th>
<th>Time</th>
<th>Standardized time (St)</th>
<th>$Sv \times St$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Braun-Blanquet</td>
<td>0.276</td>
<td>1.84</td>
<td>7.37</td>
<td>2.21</td>
<td>4.07</td>
</tr>
<tr>
<td>Grid percentage</td>
<td>0.209</td>
<td>1.39</td>
<td>23.90</td>
<td>7.19</td>
<td>10.00</td>
</tr>
<tr>
<td>Digital image</td>
<td>0.155</td>
<td>1.00</td>
<td>3.33</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>
image method, though its time investment falls within the range of the grid percentage method, consumes longer time in the laboratory. Certainly, the most valuable advantage of the digital method is that the original data source can be revisited in case of need, lost data, outliers or identification of new variables in the original data (e.g., algae cover, detritus accumulation, colonization).

Some of the results showed that the estimation of cover using the Braun-Blanquet method was more reliable than using the grid method; however, the former presented lower precision and higher error in the regression with the dry weight. The higher error is mainly due to the difficulty of assigning precise values that are lower than 10% of the cover and thus likely to generate an over-dispersion of the points. However, the Braun-Blanquet method has the advantage that it requires a minimum time in the field (although not as low as the digital image method) and its application to large areas. Studies done in the southern boreal coniferous zone of Sweden showed the visual estimation of species abundance could be obtained rapidly. However, this required a prior calibration of the different observers in order to reduce the inter-observer variation (Vanha-Majamaa et al. 2000).

The image processing technique for estimating species cover was proposed as both an objective method and one resulting in low variability among data gathered by observers (Price et al. 1993; Vanha-Majamaa et al. 2000). By contrast, the Braun-Blanquet and grid methods are visually-based. They are therefore more subject to undesirable data variation resulting from observers. Neither frequency analysis nor automatic methods have these flaws (Carlsson et al. 2005).

However, the particular techniques of image processing may be affected by choices, including image resolution and the fact that the system setup must be determined before the study (i.e., classification can be affected by unsupervised vs. supervised classifications of the images in the GIS software or by the particular algorithms used to obtain the cover estimates; Vanha-Majamaa et al. 2000).

The method has an additional drawback: that of species recognition. This can be partially solved by using high resolution images and improved illumination (e.g., the variation in tones from a single species may be superimposed upon those from the surrounding species and thereby generate a confusing signature). The changes in a single plant, from older to younger parts, may introduce as much variation as that observed between a dark-colored species of *Pleurozia* or *Ceratolejeunea* and a light-colored *Dicranum* or *Leucobryum*. The processing of digital images also has a low efficiency in multilayered vegetation where the layers are superimposed and the upper layer does not allow the lens to capture the information behind it (Vanha-Majamaa et al. 2000). However a modification of the method may be employed to characterize the complexity of the stratification by taking pictures from the sides of the plot, extracting the profiles of the vegetation and then estimating the cover of the different layers (Zehm et al. 2003). An additional issue concerns digital image storage. Indeed, after images are extracted from compressed picture formats (i.e., JPEG image files) their size is three to four times that of the original. Every successive image will have a larger size making the final files from a single picture nearly ten times larger than the original file.

One important application of the method is its capacity to monitor bryophyte dynamics through the GIS software. This function stems from its using reference points to attain a very precise superimposition of images. Depending upon the methodology utilized, the sampled areas require several reference points (4–9). The points, demarked with reflective pins, should be clearly visible and can be located in the corners of the plots. Using the points, an accurate superimposition of the images can be obtained while also allowing the user to detect minor variations in the species cover as assessed on different dates (Benavides 2007). We have also employed it, using high resolution images (i.e., over 10 megapixels), in cover estimation of small epiphylls. The method allowed cover estimation of small-statured species such as *Aphanolejeunea gracilis* and *Leptolejeunea tridentata*, both of which are less than 2 mm wide (Benavides & Sastre-De Jesús unpublished data).

A powerful tool on GIS image managements is the development of techniques to project images with askew layouts in a common frame. Specific modules,
such as the Resampling module in IDRISI, allow one to project an image in a common framework. This is achieved by identifying common reference points (control points) between the image to be projected and the common framework (Eastman 2003). In a multi-temporal study on bryophyte dynamics using successive images of bryophytes growing on clay tiles for several months, the common frame. In addition, a reference image was obtained from a completely horizontal tile. The corners of the tile were used as the reference points and all the images were rotated and transformed to fit this original, horizontal tile. The efficacy of this projection depends on the degree of deformation of the target image. The problem is overcome through increasing the number of control points and selecting more sophisticated geometric transformations that fit non-even spherical or cylindrical deformations.

The digital method described herein can substitute destructive sampling while providing an alternative measurement of cover characterized by low variation attributable to data obtained from different observers, high reliability, and high efficiency. We suggest implementing this technique by using the highest possible image contrast between the bryophyte species and their background. This can be done using lateral flashlights or avoiding shadows in the plot area.

One important factor not considered in this study is that of the observers’ mental fatigue after several cover estimates. The visual estimates (e.g., those made with the Braun-Blanquet and grid methods), conducted in the field and under non-optimal conditions, have a higher probability of imposing mental fatigue upon the observer than those of the digital image method (Bräkenhielm & Qinghong 1995).

**Acknowledgements**

We express our gratitude to Jenny Urbina and Rafael Prieto for their collaboration as observers. Also we thank two anonymous reviewers for their comments to the paper. Collections from the Toro Negro and Guajataca Commonwealth Forests of Puerto Rico were carried out under a collecting permit issued to Juan Carlos Benavides by the Puerto Rico Department of Natural Resources.

**Literature Cited**

Anonymous. 1994. ERDAS Field Guide. ERDAS, Inc., Atlanta, GA.


R Development Core Team. 2006. R: A language and environment for statistical computing. R Foundation for
ms. received September 17, 2007; accepted April 16, 2008.