A Nondestructive Image Analysis Technique for Estimating Whole-tree Leaf Area

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Summary. The development of a rapid, accurate, yet nondestructive technique for expressing whole-tree leaf area would be extremely useful in studying various growth phenomena in trees. The objective of this research was to evaluate the accuracy of an image analysis process adapted for estimating the leaf surface area of four broad-leafed tree species (*Amelanchier* L. 'Robin Hill Pink', *Tilia americana* L. 'Redmond', *Sophora japonica* L. 'Regent', *Fraxinus americana* L.'Autumn Purple' and *Fraxinus pennsylvanica* Marsh.). Video images of photographs taken of each tree canopy were quantified by an image analyzer into unitless surface area values or silhouette areas. The relationship between estimated leaf area as calculated from silhouette area and actual leaf area of these trees as determined by a leaf area meter was highly correlated. Use of this technique would enable a researcher, simply from serial photographs of the canopy, to retroactively estimate leaf or canopy area at crucial interim periods.

The study of various growth phenomena and canopy exchange processes in trees often requires repeated estimations of leaf area over time. No one method presently offers the researcher a quick, nondestructive, and yet accurate pro-

cess for quantifying leaf area at interim periods of growth during the course of an experiment.

Current nondestructive methods for determining leaf areas of woody plants are few and include: defining a mathematical relationship between the length and/or width of a leaf laminae and leaf area (Ackley et al., 1958; Sepulveda and Kliewer, 1983); foliar area estimated by regression with sapwood cross-sectional area (Rogers and Hinckley, 1979; Waring et al., 1982); correlating shoot leaf area with stem length (Johnson and Lakso, 1985); correlating the volume of plant shoots obtained through water displacement with either fresh plant weight (Burdett, 1979; Johnson, 1983) or planimetrically traced needle area (Johnson, 1984). Unfortunately, these methods require tedious calibration for individual leaves, cultivars, or canopy types; can still require knowing the total number of leaves on a tree; or are applicable only to certain species or a specific size of plant material. Importantly, these methods offer only an indirect estimation of canopy size.

New and more promising procedures involve various image analysis systems that have been used to estimate a range of plant indices other than leaf area. Plant size, as documented photographically, was highly correlated with both fresh and dry weight of *Chrysanthemum morifolium* (Sydnor et al., 1975). The percent sky visible on hemispherical photographs of apple tree spur shoots was correlated with levels of photosynthetically active radiation in the canopy (Lakso, 1976). An attempt has been made to relate geometric volume of apple trees representing six distinct canopy forms to calculations derived from photographs digitized on a computer (Miller and Lightner, 1987). Film density was correlated with percent shade as determined by a pyranometer for five woody shade tree species (Gardner and Sydnor, 1987), and used to determine meaningful differences in winter crown density for three other tree species (Wagner and Heisler, 1986).

In all of the above studies, the images were analyzed using computer-controlled scanning microdensitometers, which enable the user to digitize the photographic image into isolated moments or pixels. While this system

was used previously to quantify root length (Vorhees et al., 1980), recent and more sophisticated variations have been used to determine percent groundcover of crops from overhead slides (Thomas et al., 1988), to quantify growth of micropropagules in vitro (Smith et al., 1989), and to evaluate tree canopy color (Headley and Mower, 1990). These systems, because of their complexity, are quite expensive and usually are custom-developed for specific applications. While they do allow further manipulation and resolution of the image, especially when coupled to image analysis software, it is not clear that these additional capabilities are needed for a determination of leaf area.

A simpler, less-expensive commercially available scanning microdensitometer (the Delta-T Devices Area Meter, Decagon Devices, Pullman, Wash.) is not computer-driven and does not have digitizing capabilities but seems to hold great potential. The Delta-T consists of a video monitor coupled to an area meter that processes the signal (image) received by a black-and-white video camera. Simply, a video image is taken of a tree (in this case a photograph is used) and then transferred to the monitor. When the area meter is set in the area mode, the image is scanned line by line. A threshold level then is set by the user. This determines the gray level at which the fraction of every line in a scanned image reads as either black or white. A schematic diagram of the process described in this paper is shown in Fig. 1.

By manipulating the threshold, the user recreates on the monitor a scanned image of the tree that is superimposed over the original video image of the tree. Feedback as to the accuracy of this re-created image is immediate because the result of each threshold setting can be confirmed visually on the screen (Fig. 2). A counter totals the scanned line fractions to give the percent of the total viewing field that is composed of tree canopy. This percent density is a unitless quantification of the area of the object on the screen and has been labeled the silhouette area (SA) by Carter and Smith (1985). The term SA evolved from an understanding that, in a three-dimensional canopy, certain portions of leaf area will remain "unseen" due to leaf overlap, resulting in an underestimation of actual leaf surface area, while inclusion of the stem results in an overestimation.

The Delta-T more commonly has been used with the area meter set in the length mode to obtain root length measurements of herbaceous and woody seedlings (Barnett et al., 1987; Cunningham et al., 1989; Harris and Campbell, 1989). However, if set in the area mode, Diebolt and Mudge (1988) found that when small *Pinus sylvestris* L. seedlings were videoed

directly, the resulting silhouette area was correlated highly with more conventional methods of indirectly estimating needle surface area, such as needle dry weight and volume displacement. In the study above, silhouette area was related to actual leaf surface area by using wires and paper squares of known area in the viewing field. Using just the unitless silhouette area values, Green and Watson (1989) attempted to distinguish significant differences in crown development among 6.25-cm caliper *Acer saccharum* Marsh. trees.

This paper compares current procedures for calculating leaf area and describes a process for calculating the two-dimensional leaf area of an intact tree using photographs and the Delta-T video imaging system. This image-derived leaf area was compared with values obtained by passing the leaves through a leaf-area meter.

Materials and methods

The method entailed three steps: 1) photography; 2) image processing, from which leaf area is estimated; and 3) determination of actual leaf area with a leaf-area meter. A total of 150 trees were used to evaluate the effectiveness and accuracy of this image-analysis system. The species represented a range of mean areas per leaf, leaf arrangement, and canopy size. Because of differences in canopy height, however, the trees were divided into two
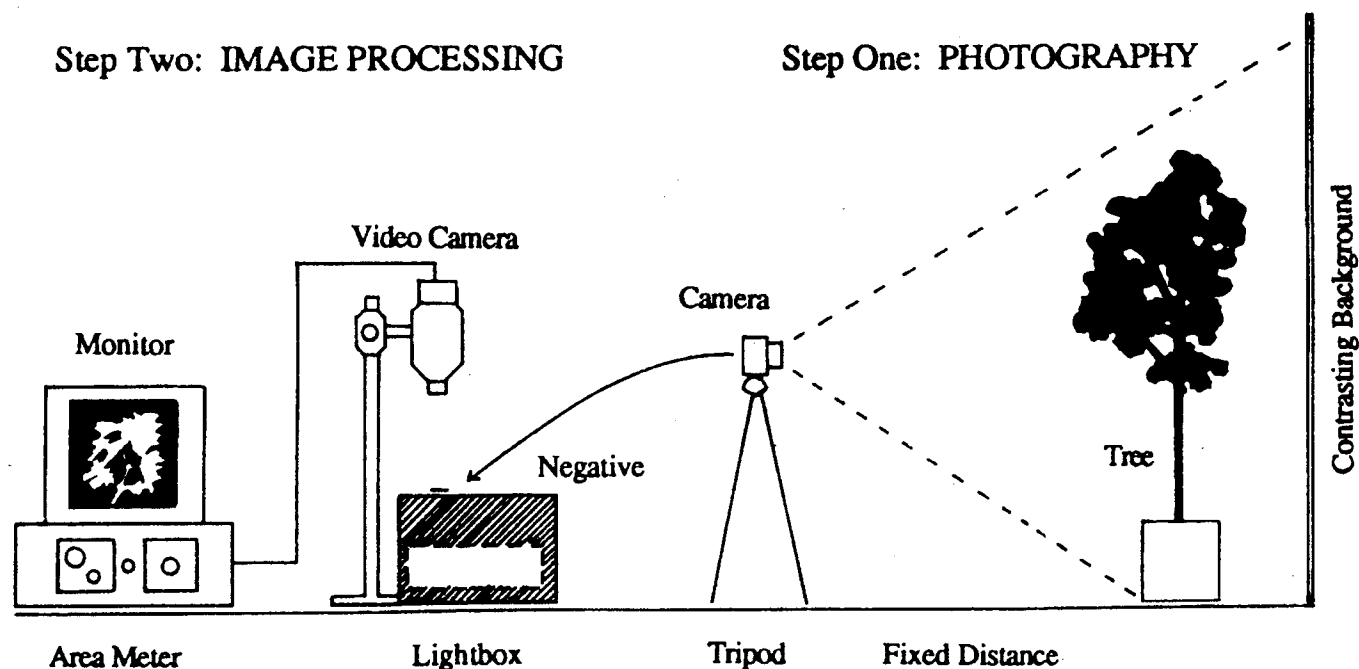

Step Two: IMAGE PROCESSING   Step One: PHOTOGRAPHY

Monitor   Video Camera   Camera   Tree   Contrasting Background

Negative

Area Meter   Lightbox   Tripod   Fixed Distance

Fig. 1. *A schematic diagram of the image analysis technique for determining the leaf surface area of a tree canopy. Not to scale.*

Fig. 2. *A serial of the scanned image of an ash tree as the appropriate threshold level is achieved. (Left) 100.0% white (percent shown at the top right side of each frame). (Middle) As the threshold is decreased, less of the background image reads as white.(Right) Just the actual tree canopy is illuminated by scan lines (representing 33.3% of the entire frame).*

groups for photographing. The distance between the camera and the tree must be kept constant to maintain a consistently sized photographic frame from which to estimate actual leaf surface area (explained further in the section on scaling). Group two trees had less height, and the camera was moved closer to the tree to capture more fully the canopy in the photographic frame.

Group one consisted of 54 trees, 18 trees each (10- to 40-mm stem caliper, 1.4 to 2.5 m high) of *Amelanchier*'Robin Hill Pink'/serviceberry, *Tilia americana* 'Redmond'/linden, and *Fraxinus americana* 'Autumn Purple'/white ash. Group two consisted of 96 trees, 48 trees each (15- to 20-mm stem caliper, 1 m high) of *F. pennsylvanica*/green ash and *Sophora japonica* 'Regent'/pagoda tree. General leaf and canopy parameters for each species are given in Table 1. For illustrative purposes, leaf shapes and canopy forms for Group one are shown in Fig. 3.

Group one

The photographic frame for Group one trees was set up to accommodate the largest tree canopy. This distance, 4 m from the camera lens to the outer facing edge of the tree container, was kept constant. Each tree was photographed in full leaf four times, turning the container 90° while in place, against a 2.5 × 5-m-wide sheet of Widetone Photographic paper in an indoor area with ceiling-mounted fluorescent lights. A 35-mm Canon AE-1 programmable camera with a 50-mm, 1.8 lens mounted on a tripod was used. To accommodate the low indoor light levels yet maintain an adequate depth of field, an f-stop of 5.6 was maintained as a constant by manipulating shutter speed. The film used was Kodak Tri-X Pan, 400-ASA black-and-white negative film selected for its fine grain and medium- to high-contrast capabilities.

All film was processed into negatives and prints using recommended Kodak development and printing techniques and materials. Kodak Microdol-X developer was selected as a fine-grain developer that produces excellent resolution of detail (Kodak, 1976). The negatives then were enlarged by 98% into a 12.5 × 17.5-cm format and printed onto Kodak Kodabromide Resin Coated f-5 grade paper, which allows small, dense negatives to be enlarged to high magnification without a resulting loss of resolution (Kodak, 1973).

Group two

For the second group of trees, Kodak Technical Pan 2415 black-and-white film set at ASA 100 was used. This film was selected because of its extremely high-contrast abilities and fine grain, even as compared with Tri-X Pan. Tech Pan was designed as a film with high-contrast control, especially useful for bringing out low-contrast subjects (Gardner and Syndor, 1987; O'Neill, 1984). The negatives were developed according to Kodak specifications using D-19 developer, noted for having a high-contrast index (Kodak, 1976). Prints were not developed for this group–only the negatives were analyzed. The distance between the camera and each tree was kept constant at 2.8 m. The setup was as described for Group one trees.

Table 1. *Leaf and canopy parameters for five tree species.*

Tree	Stem caliper (cm)	Ht (m)	Overall canopy area (cm²)			Mean area/ leaf (cm²)
			Min	Mean	Max	
Group one						
Amelanchier Robin Hill Pink	1–4	1.8–3	1,909	7,466	15,343	10.91
Tilia americana Redmond	1–3.1	1.4–2.5	2,331	5,263	7,543	46.62
Fraxinus americana Autumn Purple	1.2–3	1.4–2.4	2,284	10,170	15,197	14.24
Group two						
Sophora japonica Regent	0.68–0.8	1	666	2,346	4,347	1.24
F. pennsylvanica	0.6–0.8	1	1,246	3,112	5,901	10.44

Amelanchier 'Robin Hill Pink'   *Fraxinus americana* 'Autumn Purple'   *Tilia americana* 'Redmond'
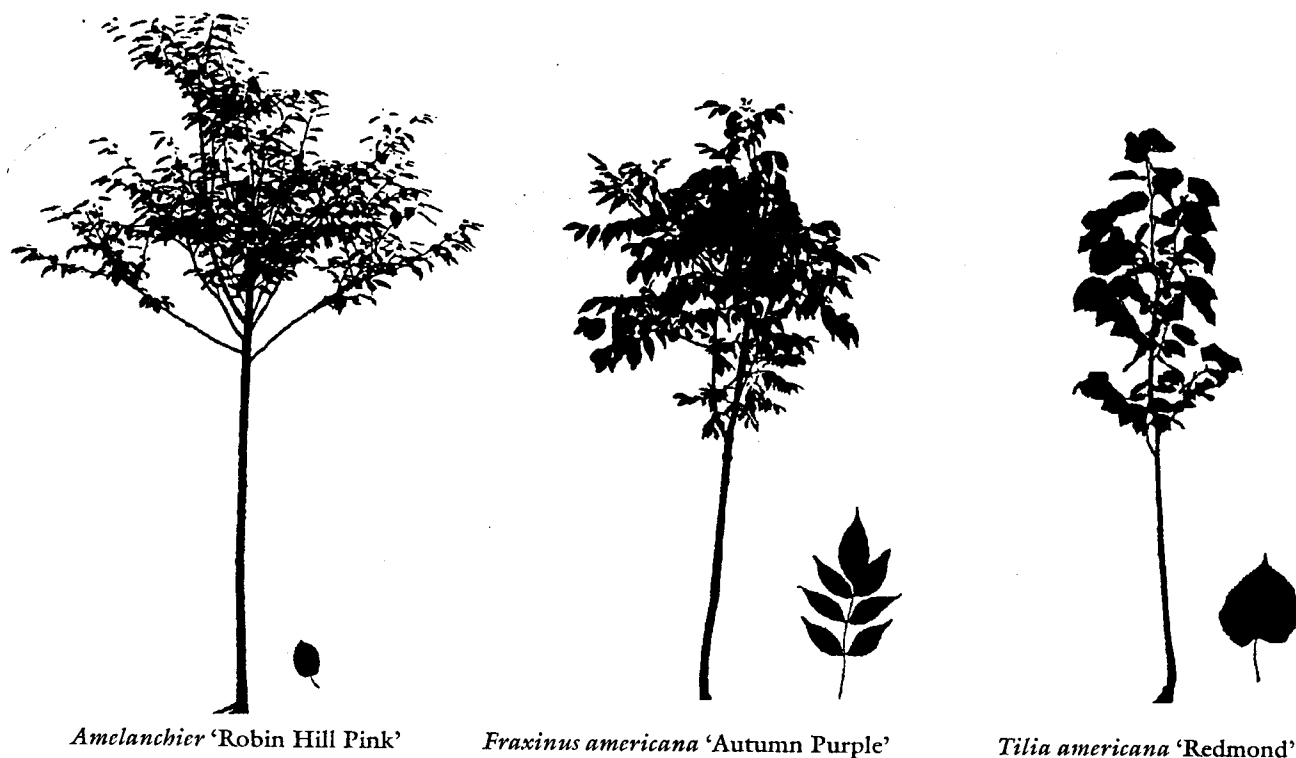
Fig. 3. *High-contrast photographic images of each species in Group one, along with the leaf/leaflet of each. Tree silhouette and leaves are not to scale.*

Results

Each photographic image for both groups of trees was then analyzed. The Delta-T Area Meter was linked to an RCA (TC1 115) black-and-white video monitor and an RCA (TC 2011/U) 18-mm vidicon camera mounted with a Nikon 55-mm, f-2.8 micro lens (Fig. 1). The camera was mounted on a copy stand and directed downward to a 41 × 61-cm light box containing two rows of double-tubed fluorescent lights. Illuminating the photographs and negatives from underneath was necessary to achieve maximum contrast between the tree canopy and the background, maximizing the detection of the canopy outline. To diffuse the light and eliminate glare, a sheet of chromatography paper was placed between the double-paned glass. Both the negatives and the prints then were mounted directly on the light box perpendicular to the video camera lens.

The Delta-T meter readout was set to the area mode and calibrated to give a percent reading. Additional front panel controls allowed setting of the measurement period and a multiplier ($\times 2^n$), both of which adjust the readout by factors of two in order to give an area readout of 1000 (e.g., a mea-

sured reading of 0175 really means 17.5%). For negatives the threshold level was set at the extreme end to read white at 1000 (100.0%). This setup assumed a transparent photographic image of a "white" tree canopy against a "black" background. The f-stop for the video camera was set at 8, the aperture that seemed to best accentuate detail, as confirmed visually on the monitor image. A threshold level was established initially with one negative and is the one that resulted in most if not all of the photographic image being matched with overlaid scan lines (Fig. 2). This threshold level then was held constant for all of the negatives. Precise focusing at this point was crucial for maximum resolution. The SA then was recorded from the digital readout. SA is the quantification, on a percent basis, of the amount of white (in this case leaf area) that is being viewed on the screen. The prints were processed similarly to the negatives. However, the readout was calibrated to read black at 100.0%. This procedure was set up to read a photographic image of a "black" tree canopy against a white background. The mean SA from four readings per tree, from both negatives and prints, then was calculated.

In the final step, each of these mean SA values that were derived both from negatives (Groups one and two) and prints (Group one) must be converted to a value expressing the actual life-size canopy. It is necessary to estimate then how much actual leaf surface area is displayed on the monitor screen. The following calibrations enable the conversion of the unitless percent SA number given by the Delta-T into the quantity of estimated leaf-surface area being viewed.

Scaling from the negative for Group one trees. A) The scale of a negative taken with a 50-mm lens is calculated as the representative fraction (Avery, 1977).

$$\frac{\text{Camera focal length (50 mm)}}{\text{Distance from camera to tree}} = \frac{5 \text{ cm}}{406 \text{ cm}}$$

Both are then divided by 5 cm to yield the relationship

1 cm on the negative = 81.28 cm in reality (1:81.28)

B) The negative then is placed on top of the light box with the video camera recording the image and projecting it on the monitor screen. Now that we have an image on the monitor,

how do we scale this image? A square frame that captures all of the canopy is drawn with a black marker on the glass surface of the light box (directly under the negative). As much of the trunk area in the negative as possible is eliminated. These frame dimensions (1.1 × 1.5 cm) then were multiplied by the value established in step A to obtain the actual area inside the photographic frame of the tree.

Frame: $[(1.1 \text{ cm} \times 81.28) \times (1.5 \text{ cm} \times 81.28)] = 10,886 \text{ cm}^2$

C) To now derive the actual leaf surface area from the percent SA reading, SA was multiplied by the actual frame area as calculated above. For instance, if a mean SA of 43% was obtained for a tree, 10,886 cm² is multiplied by 0.43 to obtain 4681 cm² of estimated actual leaf surface area.

Scaling from the negatives for Group two trees. Negatives were scaled in a similar manner except that the distance from camera to tree was reduced for the smaller trees, as noted previously

$$\frac{\text{Camera focal length}}{\text{Distance from camera to tree}} = \frac{5 \text{ cm}}{287 \text{cm}}$$

1 cm on negative = 57.4 cm in actuality

Frame: $[(1.7 \text{ cm} \times 57.4 \text{ cm}) \times (2.3 \text{ cm} \times 57.4)] = 12,882 \text{ cm}^2$

To derive the leaf surface area for a given tree, this value, 12,882 cm², is multiplied by the mean percent SA.

Scaling from the prints. Only Group one negatives were printed to paper and subsequently analyzed. To scale from the negatives to the prints, an object of known size on the negative (in this case, the width of the growing container) was compared with the same object on the print. A 1:5 enlargement ratio was found. The frame dimensions traced on the light box for the larger prints (13.6×9.8 cm) then were divided by 5

13.6 cm ÷ 5 = 2.7 cm
9.8 cm ÷ 5 = 1.96 cm

To derive the total area represented inside the photographic frame, the frame dimensions then are multiplied by the 81.28-cm enlargement value obtained in Step 1.
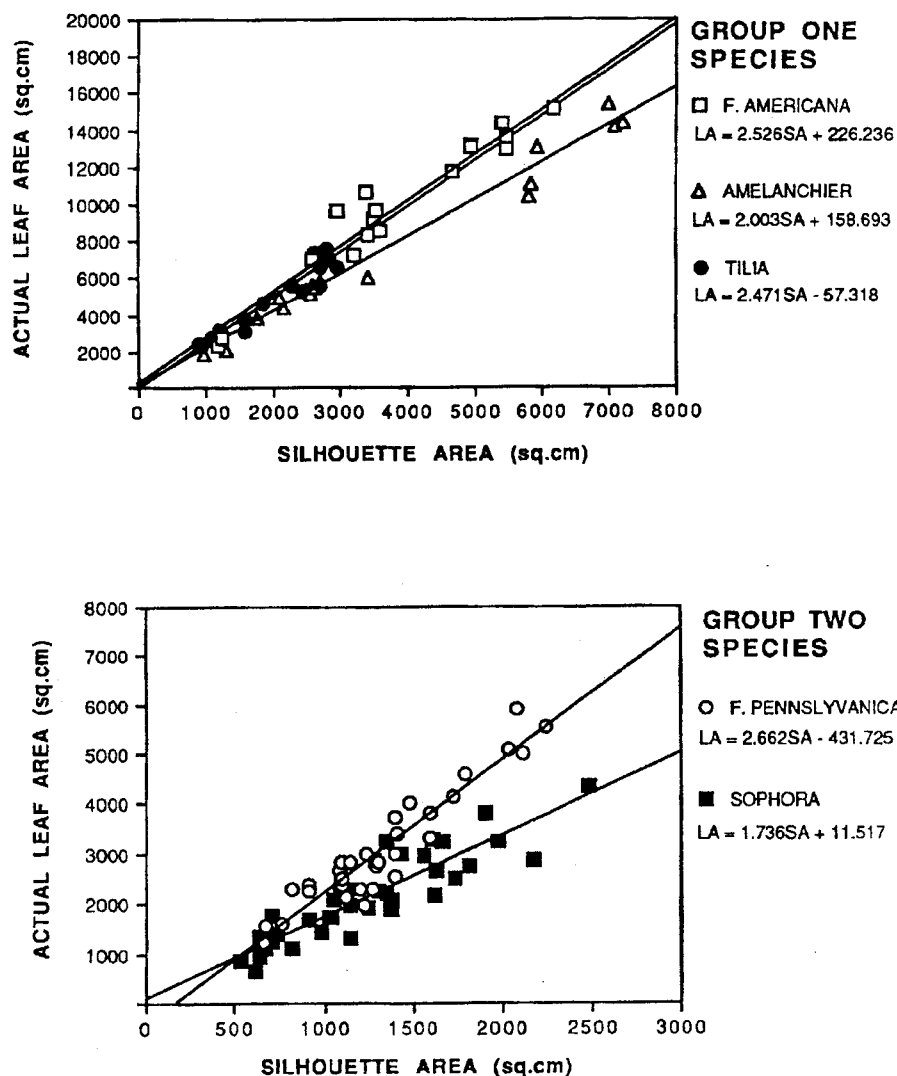

Fig. 4. *Graphs of the linear relationship between silhouette area (SA) and actual leaf area (LA), so that LA = m(SA) + b, where m = slope of the line and b = y intercept. Each regression was significant at P ≤ 0.01. The difference in Group one and Group two trees is the canopy-to-camera lens distance, 4 and 2.8 m, respectively.*

2.7 cm (81.28 cm) × 1.96 cm (81.28 cm) = 35,219 cm²

To calculate the estimated leaf surface area for a given tree from the photograph, this value, 35,219 cm², is multiplied by the mean percent SA.

Each of the trees then was stripped of all leaves, and the leaves were passed through a LI-COR model 3100 Leaf Area Meter (LI-COR, Lincoln, Neb.) to obtain the total actual leaf surface area.

Results and discussion

The use of silhouette area as an estimator of actual leaf area. For all five species, a simple linear regression model was determined, actual leaf area (dependent variable y) being regressed

with SA (independent variable x) to fit a straight line (Fig. 4). The regressions were derived from SA values expressed in square centimeters and representing the mean of four negatives of each tree. All the regressions exhibited a highly significant linear relationship between SA and leaf area for each species, indicating that SA is an excellent estimator of actual leaf surface area for these species within the range of canopy sizes examined, 600 to >15,000 cm² of leaf surface area.

The use of prints vs. negatives. If negatives are as accurate as high-contrast prints in the SA values obtained, it would be much less time-consuming and more efficient to eliminate the printing-to-paper step in the development process, and just use negatives.

Table 2. *Analysis of differences for Group one trees between using either prints or negatives. In regressions, leaf area (y) is regressed against silhouette area (x)[z].*

Species	Regression with 4 negs.[y] (R^2)	Regression with 4 prints[y] (R^2)	Correlation of mean of 4 prints vs. mean of 4 negs. (r)/tree	Regression with 2 negs.[x] (R^2)
Amelanchier	0.977	0.942	0.987	0.977
Tilia	0.920	0.945	0.992	0.905
F. americana	0.942	0.870	0.895	0.922

[z]*Each regression and correlation was significant at* $P \leq 0.01$, $n = 18$ *trees.*
[y]*Calculated using the mean of four photographs (negatives or prints as noted) taken at 90° from each other.*
[x]*Calculated using the mean of two negatives taken at 90° from each other.*

To test whether the difference between prints and negatives was significant in terms of the SA readings obtained, SA values derived using the mean of four prints per tree were regressed on actual leaf area, and the strength of this relationship was compared with the original regressions run with the mean of four negatives per tree for Group one trees (Table 2). A correlation then was established between the SA values obtained with prints vs. negatives.

For *Amelanchier, Tilia,* and *Fraxinus,* the regressions were still highly significant using prints. In the case of *Amelanchier* and *Fraxinus,* the use of negatives is justified over the use of prints, with higher R^2 values being obtained for the former. In the case of *Tilia,* the higher R^2 is obtained for prints, $R^2 = 0.94$ vs. 0.92. However, the correlation coefficient between sets of the two values is very high at $r = 0.992$, which means that the difference is not significant and that either prints or negatives could be used.

Determining the appropropriate number of photographic images needed. In the interest of further simplifying the process, it is important to know the minimum number of photographs needed to obtain a SA value still significantly correlated with actual leaf area. Since photographs taken 180° apart should produce almost identical silhouette values, it would seem that just two photographs taken 90° apart would be sufficient. Miller and Lightner (1987) took 16 photographs at equal points around the perimeter of an apple tree canopy and determined that two photographs taken 90° apart were sufficient to compare with a geometrically derived canopy volume.

Group one trees were used for this analysis. For each tree the mean of two negatives taken 90° apart was compared with the mean of all four negatives for each tree (Table 2). For *Amelanchier,* the R^2 values (0.97) are identical whether using two or four negatives taken 90° apart. For *Tilia* and *Fraxinus,* the proportion of variation in leaf area that can be attributed to a linear association with SA using just two negatives is a little less, 2%. These are more than acceptable tolerances in accuracy, given the increased efficiency in having to take, process, and analyze only two photographs per tree. The technique can be simplified by using only two negatives of each tree shot at 90° apart.

Analysis of species differences

Were there optimal leaf/canopy sizes and shapes at which SA was a better predictor of actual leaf area? While the relationship between SA and leaf area is significantly linear for each species, it is not 1:1 for any of the species. For all species, SA represents only a certain proportion of actual leaf area. This is intuitive, as one would not expect a two-dimensional image to capture fully a three-dimensional canopy volume. A term, introduced here to quantify this proportion, is referred to as "photographic effectiveness" or PE, which is, in effect, the slope of the regression line expressed on a percent basis. This relative change in leaf area for every 1-cm change in SA is a function of 1) capturing only varying fractions of each whole leaf in the picture frame, depending on its orientation; and 2) the amount of leaf overlap that occurs as a function of leaf size and tree architecture. In this case, PE was an indication of the degree to which SA "underestimated" actual leaf area.

Photographic effectiveness for *Amelanchier* and *Sophora* was high, 50% and 57%, respectively. Both species had small leaves that angled slightly downward well spaced along the branches with minimal overlap as compared with the other species. Branching patterns were generally upright with the overall canopy form being quite loose and open, resulting in a higher percentage of actual leaf surface area being photographed. Also, the inclusion of some stem area in the photograph would tend to raise the PE value, with both of these species presenting a larger stem : leaf area ratio than the remaining species.

While *F. pennsylvanica* also had a small leaflet, its canopy form was not nearly as loose and open as any of the preceding species. Its PE was low, 37%, which means that 26% to 35% less actual leaf for each tree was presented two-dimensionally. Since the leaflets folded slightly along the petioles to form a V-shaped leaf extending slightly upward, this effectively could have halved the photographed leaf area for many of the leaves.

Both *Tilia* and *F. americana* had large leaves that generally hung down and faced outward and that were clustered more densely around the stem and over the top of each other. They had PE values in the middle range, 40% and 39%, respectively. A test of significance indicated that the slopes of *Tilia* and *F. americana* were not significantly different from each other ($P \leq 0.05$)

The differences in PE seemed to be influenced mostly by canopy architecture, density, and leaf angle and orientation. Generally it can be said that loose, open canopies with small leaves result in higher PE values, while trees with larger leaves that hang down or fold and are clustered more closely together (with significant overlap) result in lower PE values. The values obtained in this project are in keeping with PE estimated from the data of Diebolt and Mudge (1988), in which there occurred a slightly lower PE, 35%, for narrow-needled Scots pine seedlings (R^2, 0.95).

For each species, there existed a constant additive factor (the intercept value) that represented some error in addition to the proportionality relationship. High positive values of this factor might be attributed to any of the following factors: insufficient depth of field and hence resolution of the canopy by the camera and video lenses (equipment resolution limits) and poor contrast between the tree canopy and the

background. An f-stop of 5.6 was used to photograph these trees, a trade-off to adjust for low natural-light conditions, when higher f-stops of 16 to 22 would be better-suited to obtain sharper images of a three-dimensional canopy. The video camera was set at f-stop 8, which seemed to best accentuate the detail, and there was visual confirmation of this directly on the area meter monitor. Potentially, if resolution was poor, intercepts would be higher for the more finely textured species, since they presented a more finely scalloped silhouette and more intracanopy open space than did the coarser-textured species, which was not the case.

Conclusion

A significant relationship must exist between actual leaf area and SA to use the latter as an accurate and nondestructive estimator of actual leaf area. This study established that two negative images of the canopy taken 90° apart with a high-contrast film and analyzed on the Delta-T video imaging system were adequate for establishing the necessary predictive equation. This image analysis technique was valid for species representing a range of leaf sizes, overall canopy areas, and tree architecture types. Importantly, it is nondestructive, allowing for an accurate assessment of actual leaf area or even, more simply, crown growth at critical interim periods during a research project. In addition, it may be a critical tool when quick tree growth response needs to be documented.

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