Comparison of methods for estimating surface area of water-stressed and fully hydrated pine needle segments for gas exchange analysis

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Summary
A nondestructive technique for repeatedly estimating total surface area of water-stressed pine (Pinus taeda L.) fascicle segments was evaluated. Fascicle radius was measured with a digital caliper and needle surface area calculated on the assumption that the needles were segments of a cylinder. High correlations (r ≥ 0.94) were found between surface area estimated in this way and surface area calculated (a) from needle displacement, also on the assumption that needles are segments of a cylinder, and (b) from needle dry weight using a regression between dry weight and surface area estimated from needle displacement. The caliper-radius method gave slightly lower estimates of surface area than the other methods. However, differences among surface area estimates made with the three methods and between estimates made on water-stressed and fully hydrated needles were small relative to variances of leaf gas exchange measurements.

Introduction
Leaf surface area is a primary variable for many forms of forest productivity research. The leaf area of broad-leaved trees or of conifers with flattened needles can be estimated with a leaf area meter. The leaf surface area of many conifers cannot be estimated accurately, however, by considering the needles as though they were two-dimensional. With these species the glass-bead coating method (Thompson and Leyton 1971) can be used, but it is labor-intensive. Models of needle geometry have been used to estimate needle surface area either from needle displacement and length (Johnson 1984, Brand 1987), or from needle radius and length (Fites and Teskey 1988). However, techniques based on constant geometric models are prone to error, because of changes in needle geometry with needle hydration. Needle surface areas have been estimated from regressions of foliage mass to area (Ohmart and Thomas 1986), but treatment-induced changes in specific leaf area may introduce errors (Shelton et al. 1984).

All of the previously described techniques for estimating leaf area require removal of foliage from the plant (Kvet and Marshall 1971, Johnson 1984), which precludes repeated measurements of the same leaf. Because much research on pines involves
studies of gas exchange and water stress, a rapid, nondestructive technique that is independent of environmentally induced changes in needle morphology is desirable. The objective of this research was to compare a rapid, nondestructive technique for estimating surface area of fascicle segments with two destructive sampling techniques.

Materials and methods

Two open-pollinated families of *Pinus taeda* L. were used: an East Texas provenance (Texas Forest Service 174/176) from a seed orchard in Magnolia Springs, Texas, USA, and a Louisiana provenance collected from Livingston Parrish, Louisiana, USA. Neither provenance is considered to be drought hardy.

Thirty-four-week-old seedlings growing in 1-liter containers were subjected to: (1) a high-water regime in which seedlings were watered daily to container capacity (predawn $\Psi_{fascicle} = -0.5 \pm 0.1$ MPa); and (2) a low-water regime in which seedlings were watered to container capacity on eleven-day cycles (predawn $\Psi_{fascicle} = -1.7 \pm 0.2$ MPa, as measured with a pressure chamber (Soil Moisture 3010)).

After the low-water regime seedlings had received five drought acclimation cycles, 16 fully expanded fascicles (at least 12 cm long) were removed at 0800 h from seedlings of each provenance × water regime combination. Samples were sealed in test-tubes containing moist filter paper and placed in a dark, refrigerated box (4 °C).

*Measurement of fascicle surface area*

Fascicle surface area was estimated in three ways: from radius, volume, and mass. Estimates from needle radius and volume were based on the assumption that needles are sectors of a cylinder (Johnson 1984) with the width of contiguous faces equal to the radius of the cylinder (Kozlowski and Schumacher 1943). Fascicle surface area (SA) was calculated by the formula $SA = 2\pi rl + 6rl$, where $r$ is fascicle radius (cm), and $l$ is fascicle length (Johnson 1984, Bengarten and Teskey 1986). Because measurements were confined to 2-cm segments of fascicles such as might be enclosed in the chamber of a leaf gas exchange measuring system, the formula simplifies to $SA = 24.57r$, so that $r$ is the only parameter needed to estimate surface area.

*Volume displacement determination*

A sewing-needle was poked into one end of a 2-cm needle segment, allowing the segment to be held steady while being entirely submerged in a graduated cylinder of 5% surfactant solution resting on an analytical balance. Based on Archimedes principle, the volume (cm$^3$) of an object is given by the weight (g) of water it displaces (Burgett 1979). The weight of water displaced by the fascicle segment was recorded to the nearest 0.1 mg. Each needle segment was measured twice, and blotted dry between measurements.

where $V$ is the measured volume, and $r = 0.69V^{1/2}$.

*Caliper-radius determination*

The radius of an interior needle with a digital caliper (Fisher Scientific) centered and at each end of each location. Microscopic technique during measurement.

*Mass regression determination*

After their volumes and mass was determined at 65 °C and their weight equation was developed for volume displacement.

*Statistical analysis*

Surface area was analyzed using a regime factorial experiment and two determinations analyzed using a 2 provenance design. All data were analyzed using a square root transformation (SAS Institute). The data were analyzed by linear regression.

*Results and discussion*

Assuming that surface area dehydrates during needle anatomical studies of Parl, low-water regime seedling techniques yielded lower surface area values for the Texas provenance) than with the other techniques obtained with the three methods. Mean differences of 12%...
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where \( V \) is the measured volume expressed in cm\(^3\).

**Caliper-radius determination**

The radius of an interior face of the needle was measured to the nearest 0.005 mm with a digital caliper (Fowler MAX-15). Radius was measured three times at the center and at each end of the needle segment, averaging the three determinations at each location. Microscopic observation revealed no needle compression with this technique during measurement.

**Mass regression determination**

After their volumes and radii had been measured, needle segments were oven dried at 65 °C and their weights recorded to the nearest 0.1 mg. A surface area prediction equation was developed from dry mass regressions on surface area calculated by volume displacement.

**Statistical analysis**

Surface area was analyzed using ANOVA for a 3 technique \( \times 2 \) provenance \( \times 2 \) water regime factorial experiment in a completely randomized design, with 48 replications and two determinations within three nested subsamples. Water potentials were analyzed using a 2 provenance \( \times 2 \) water regime factorial in a completely randomized design. All data were analyzed with SAS General Linear Models using type III sums of squares (SAS Institute, Inc., Cary, NC). Correlations among surface area estimated by differing techniques as influenced by provenance and water regime were analyzed by linear regression (Snedecor and Cochran 1967).

**Results and discussion**

Assuming that surface area of fully expanded fascicles changes little as foliage dehydrates during nonlethal water stress, an assumption that is consistent with the anatomical studies of Parker (1952), the surface area estimates of high-water and low-water regime seedlings should have been similar. However, all three estimation techniques yielded lower surface areas for water-stressed needle segments than for fully hydrated needle segments (2% less in the Louisiana provenance and 6% less in the Texas provenance) (Table 1). Thus it appears that all three methods are subject to slight error arising from variation in needle hydration.

Smaller estimates of surface area were obtained with the caliper-radius technique than with the other techniques. The difference was small, however, and results obtained with the three methods were closely correlated (Table 2).

Mean differences of 12% in leaf transpiration rates measured with five identical
Table 1. Comparison of three methods of estimating total surface area of 2-cm-long *Pinus taeda* L. fascicle segments from seedlings of two provenances grown under high and low water regimes.

| Provenance | Water regime | \( \Psi_{fascicle} \) (MPa) | \( y \) \( y \) \( y \) \( y \) \( y \) \( y \) | \( y \) \( y \) \( y \) \( y \) \( y \) \( y \) | \( y \) \( y \) \( y \) \( y \) \( y \) \( y \) | \( y \) \( y \) \( y \) \( y \) \( y \) \( y \) |
|-------------|--------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| East Texas  | High         | -0.8            | 1.90 ± 0.01 \(^2\) | 1.98 ± 0.01     | 1.99 ± 0.02     |
|             | Low          | -1.8            | 1.80 ± 0.02     | 1.87 ± 0.01     | 1.86 ± 0.01     |
| Louisiana   | High         | -0.9            | 1.91 ± 0.02     | 1.98 ± 0.02     | 1.99 ± 0.02     |
|             | Low          | -1.8            | 1.87 ± 0.02     | 1.96 ± 0.01     | 1.97 ± 0.01     |

Significance \(^3\):
- \( ** \) = significant at 0.1% level.
- \( * \) = significant at 5% level.

\(^1\) Measurements were made after five 11-day drought acclimation cycles to the low-water regime plants. Surface areas were estimated from fascicle segment dry weight; equation is in the form \( y = b_0 + b_1 \) (dry weight), with \( b_0 = 0.82 \) and \( b_1 = 1.33 \) (\( r^2 = 0.75 \)).

\(^2\) Means with standard error and ANOVA are presented; \( n = 288 \).

\(^3\) NS = nonsignificant, \( * \) = significant at 5% level, \( ** \) = significant at 0.1% level.

Table 2. Correlation coefficients (\( r \)) of caliper-radius technique with volume displacement and mass regression techniques for estimating surface area of 2-cm fascicle segments of *Pinus taeda*.

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<th>Technique</th>
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<th>Water regime</th>
<th>Correlation with caliper radius technique</th>
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<td>Volume displacement</td>
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<td>Mass regression</td>
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Steady-state porometers have been reported (Reich et al. 1988), indicating that derived/calculated physiological parameters are statistically insensitive to small variations in surface area. We conclude, therefore, that the caliper-radius technique is a reliable method for estimating the surface area of pine leaf segments enclosed in leaf chambers during gas exchange analysis.
METHODS FOR ESTIMATING PINE NEEDLE SURFACE AREA

The caliper-radius technique can be used on intact fascicles, allowing repeated gas exchange measurements on the same fascicle over prolonged periods, thereby avoiding errors associated with destructive sampling techniques. This makes the caliper-radius technique well suited for use in gas exchange analysis of intact fascicle segments enclosed in leaf chambers.

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References