Auburn University
Southern Forest
Nursery Management
Cooperative

RESEARCH REPORT 01-5

A COMPARISON OF DIGITAL SCANNERS TO MEASURE LEAF SURFACE AREA OF LOBLOLLY PINE SEEDLINGS

by
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INTRODUCTION

Nursery Coop staff have used digital scanning with WinRHIZO software (Regent Instruments Inc., Quebec, Canada) for the past several years to measure root length and surface area for loblolly and slash pine seedlings (see Research Reports 99-1 and 01-06). It has been a reliable method for measuring seedling roots. The system requires a scanner and computer, while the software provides the mathematical functions that interpret the scan and calculate root surface area and lengths. This is not as straightforward as it might seem as the software must be able to distinguish between root bifurcations and overlaps.

A series of tests were initiated to determine if this software package could be used to measure leaf (needle) surface area. Leaf surface is difficult to obtain because the standard technique requires that needles be removed from the seedling/tree and run through a moving belt scanner individually or in small groups. If a computer based scan could be used, this might result in significantly reduced measurement time.

METHODOLOGY

Twenty-five loblolly seedlings were obtained from a Coastal Plain nursery in February. The needle surfaces of these seedlings were measured twice using three techniques. All three techniques quantify square centimeters of leaf surface based on sensing the interruption of a light source. It should be noted all three techniques measure two dimensional area (i.e. one-sided). Actual needle surface area is estimated by multiplying the two dimensional number by Pi.

The above ground part of the seedling (including the stem) was placed intact directly on the computer scanner and WinRHYZO used to calculate leaf surface area - "total scan".

2. The needles were removed from each seedling and placed on the scanner and WinRHYZO used to calculate leaf surface area - "needle scan".
3 The needles removed were then measured using a leaf surface area meter (LI-3000, LI-COR® Biosciences) - the "standard" method.

Average seedling leaf areas as determined by the total scan and the needle scan were compared against the standard method using a "t" test. Also, the values for individual seedling leaf areas determined by the total and leaf scan techniques were regressed against the standard technique. Finally, the first measurement of each of the 25 seedlings was compared against the second measurement for all three techniques to determine the repeatability.

RESULTS

Both the total scan and needle scan techniques produced estimates of one-dimensional needle surface area that were significantly different from the standard technique (Table 1). Interestingly, the total scan technique produced an average value less than the standard (5.6%, based on the standard), and the needle scan produced an average value higher (8.6%), than the standard technique. Although these percentages may be considered low, they were significant, nevertheless, and it may be concluded that assuming the standard technique to be correct, the computer-based digital scans were producing erroneous values in absolute terms.

Table 1. Comparison of loblolly pine seedling leaf area measurements using different techniques.

Comparison	Average Seedling Leaf Area† (cm^2)	R^2
Total Scan vs	146.9 a	.95
Standard	155.6 b	
Leaf Scan vs	169.0 a	.99
Standard	155.6 b	
Total scan vs	147.2	
Total Scan	146.5 ns	.99
Leaf scan vs	169.3	
Leaf Scan	168.7 ns	.99
Standard vs	156.7 a	
Standard	154.4 b .99	

† Statistical separation at the .05 level indicated by different letters within a comparison.

The correlations between the computer-based scan techniques and the standard technique were very

close. Figure 2 shows the correlation between both total scan (2A) and the needle scan (2B) to the standard technique. The R^2 values (.95 and .99) indicate a very good relationship between the standard technique and the digital scan with the needle scan the best. It is apparent the accuracy of the total scan technique decreases with increasing seedling size, while the accuracy of the needle scan technique is constant through the range of seedling sizes tested. The decreasing accuracy of the total scan was probably related to the increasing amount of needle overlap on larger size seedlings and the software inability to separate individual needles. This might also account for the underestimate of leaf area compared to the standard.

One of the more interesting results of this experiment was that the standard technique was actually more variable between measurement than either computer-based method. As indicated in Table 1, the average seedling leaf area of the first seedling measurements as determined by the standard technique was significantly different than the second measurements using the same technique. This did not occur with the two computer-based scanning techniques. Regressing the first measurement against the second measurement of the same 25 seedlings, indicated very close relationships for all three techniques (Figure 2). Errors between the first and second measurement using the LICOR may be inherent (e.g. slide undetected between diodes) or related to operator error, improper calibration, or dirt on the conveyor belt. These are error sources routinely considered using this technique.

Computer-based digital scanning techniques were found to provide replicable measurements of loblolly pine seedling needle surface area. It appears likely these techniques could be used for relative comparisons of this seedling morphology parameter while significantly reducing measurement time. Obtaining total needle surface area using the standard technique requires the removal of all the needles from the seedling and then measuring (scanning) them individually or at best in small separated groups. On the other hand, the seedling shoot digital scan can calculate needle leaf area for an individual seedling in about 2 minutes per seedling. The needle scan technique requires the removal of the needles from the seedling, similar to the standard technique, but the actual needle measurement can be done in less than half the time of the standard method. Also, WinRHYZO can automatically calculate total leaf surface area of pine needles, whereas the standard technique requires the multiplication of the measured value times Pi, to obtain a total needle surface area. Further work is necessary, however, to verify the differences between digital techniques and the standard technique in terms of the absolute leaf area values.

MANAGEMENT IMPLICATIONS

This experiment has no direct application to nursery operations. The results have important implications for nursery science, however, indicating that computer-based scanning may provide a cost effective way to obtain leaf surface measurements for comparing experimental treatments.

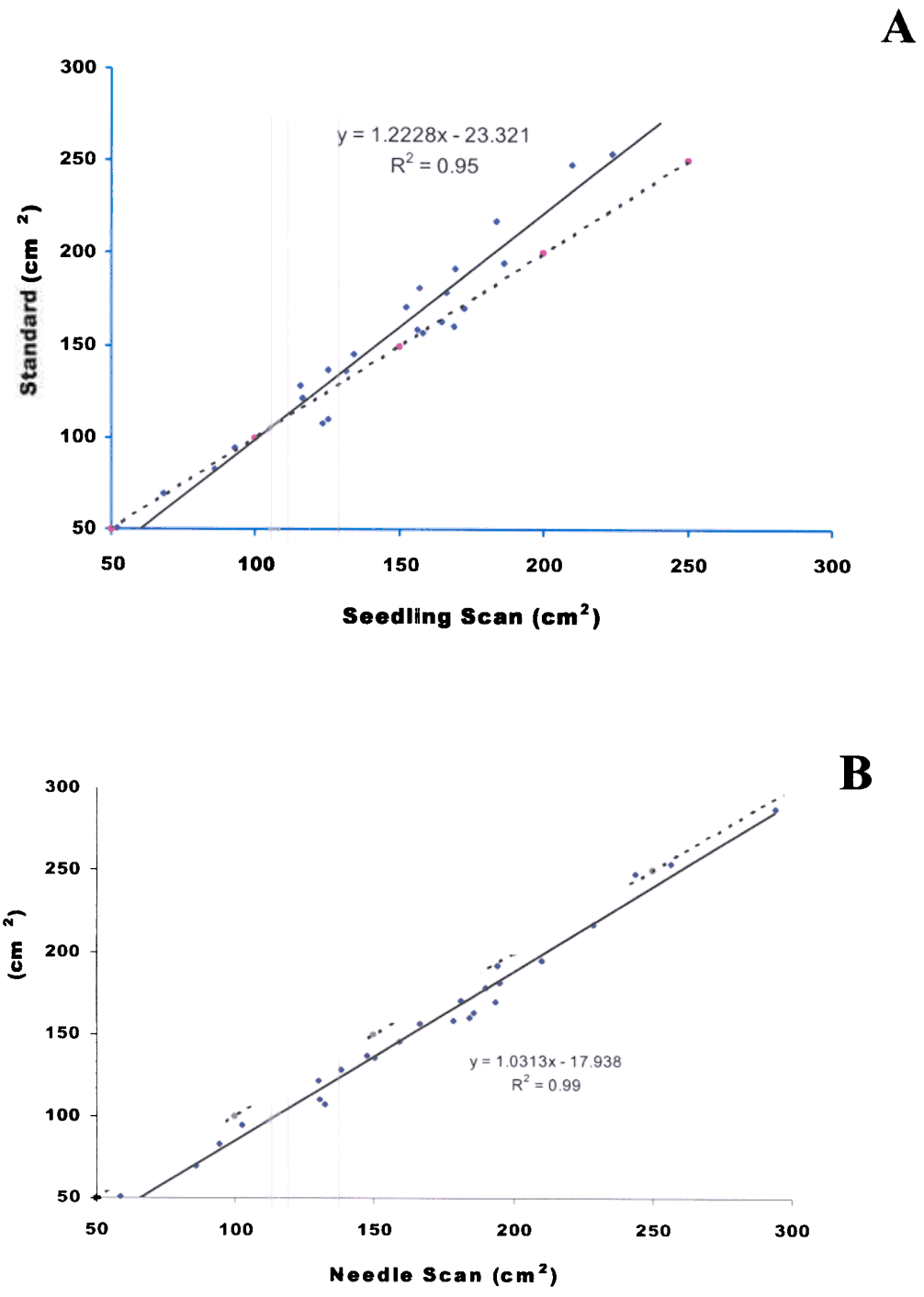
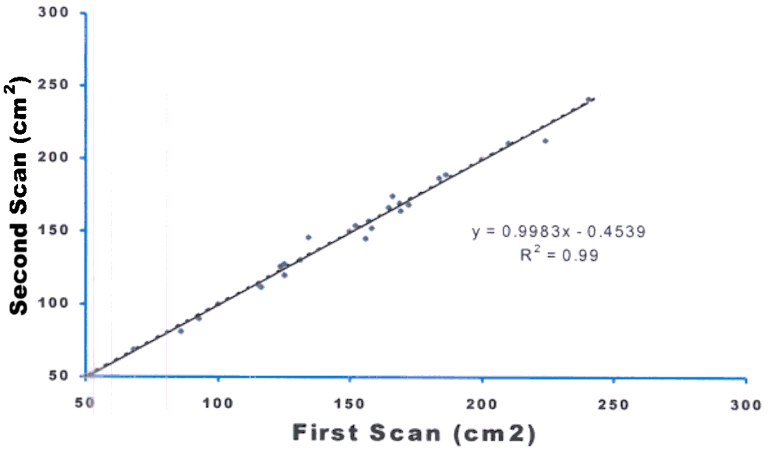
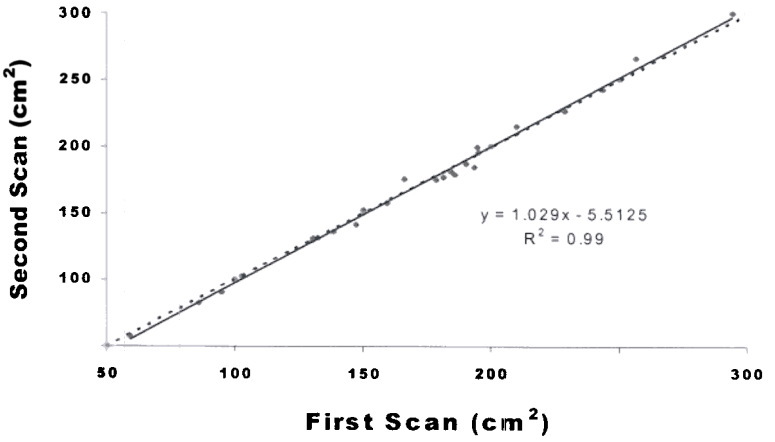


Figure 1. Plot of leaf surface area for 25 individual seedlngs measured by a scan of an entire seedling top (A) and needles only (B) scan using the WinRHYZO software package and compared to the standard leaf area index methodology. The dashed line represents a 1:1 with the standard methodology.

A
Seedling top



B
Needles only



C
Standard technique

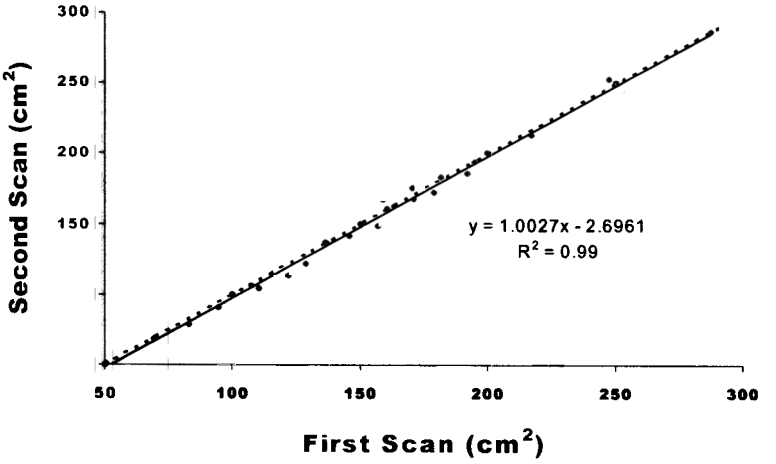


Figure 2. Regressions of the first measurement the leaf surface area of 25 individual loblolly pine seedlings against a second measurement of the same seedlings using three techniques: (A) a digital scan of the entire seedling top with LAI calculated by WinRHYZO software, (B) a digital scan of only the needles with leaf area calculated by WinRHYZO software, and (C) the standard technique. The dashed lines indicate a 1:1 correlation.