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RAPID MEASUREMENT OF FINE ROOT LENGTH USING PHOTOELECTRONIC IMAGE ANALYSIS[1]

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Abstract. Root-length estimates obtained using three different methods of a commercially available image analyzer were compared. Precision, accuracy, and the speed of obtaining estimates differed significantly among the Semirandom, Parallel, and Right Angle methods; this was a result of root orientation effects and analyzer calibration limitations. A new rapid, accurate, and precise method of obtaining root-length estimates using the analyzer was developed. The procedure interfaces the image analyzer with a microcomputer and uses a program that standardizes orientation effects during calibration.

Key words: calibration curve; image analysis; length measurement; photoelectronic image analysis; root length; roots.

INTRODUCTION

Although many studies of root dynamics have used mass as an estimate of root production and mortality (Vogt et al. 1986), both surface area and length are better indices of root function (Newman 1966). Measuring root lengths with a ruler is inefficient but improved with the development of Newman's (1966) intercept method and its subsequent modifications (Reicosky et al. 1970). Recently, photoelectronic image analyzers (IAs) with capabilities of measuring length have been developed (Rowse and Phillips 1974, Goubran and Richards 1979, Richards et al. 1979, Heringa et al. 1980, Voorhees et al. 1980, Ottman and Timm 1984, Barnett et al. 1987). Some are now commercially available, but neither their precision nor their accuracy has been adequately quantified.

Two calibration problems associated with IAs may affect their precision and accuracy. First, because hardware calibration is possible at only one scale, a linear relationship is assumed between actual root lengths and the analyzed images. If the relationship is nonlinear or lacks a 1:1 correspondence with measured values, then considerable errors result (Voorhees et al. 1980, Barnett et al. 1987). A second problem is that the object must be in clear focus, requiring positioning of the camera lens at varying heights, depending on the size of the object.

The orientation of roots on the sampling screen also can affect the accuracy of length estimates. Some IAs calculate root length using the number of intersections noted with a root present between horizontal scan lines. How a root segment is positioned will affect its estimated length. This problem may be minimized by standardizing the arrangement in relation to the scan lines, or by accounting for the effect of target orientation in the calibration procedure. As standard placement of samples is tedious, correction during calibration is preferred.

This paper describes a new method of measuring root length that standardizes effects of root orientation during calibration and is appropriate for a range of root lengths and diameter classes. Three modifications of the IA method are compared for precision, accuracy, and speed against direct measurement.

METHODS

Description of methods

The IA used consisted of an Ikegame PM930 video camera, a black-and-white TV monitor, and a Decagon Devices, Incorporated, Delta-T area meter. This IA assesses root lengths by the number of intersections between a series of horizontal scan lines and the root image on the monitor, and displays a number representing relative root length. A SONY Magnascale LM22 electronic ruler was used to measure root lengths (to ± 0.01 mm) as an empirical reference.

Three variations on IA use were compared. The Semirandom and Parallel procedures are, respectively, the length and height measurement procedures described in the manual provided with the area meter. The Right Angle method is a third technique. For the Semirandom orientation:

1) A drawing of three parallel lines (each 10 mm long for a total of 30 mm) was placed under the camera and was oriented so the lines were vertical on the monitor (perpendicular to the direction of scanning).

2) Camera height, focus, and calibration controls were adjusted until a clear image was achieved and a numerical readout value of 30 was produced.

3) Sample roots were placed on acetate sheets in a semirandom manner (long root orientation was neither 90° nor 180° to scan lines).

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4) Two readings were taken on each sample of roots, one before and one after rotating the acetate sheet 90°. These values were added to produce estimates of root length.

For the Parallel procedure:

1) and 2) as above.

3) Roots were cut into straight pieces and placed on acetate. Lengths of the root segments varied with the extent of root branching. The acetate sheet was placed so that root axes appeared vertical on the monitor.

4) One reading was taken to estimate root length.

For the Right Angle procedure:

1) Roots were placed under the camera, and the camera height, focus, and threshold were adjusted until optimal resolution for a given root diameter class was obtained. The relative value of the monitor readout number was inconsequential because a calibration curve was used to determine estimates.

2) Using a program developed to interface the IA with an Apple IIe microcomputer, a multiple-point linear-calibration curve was produced. The curve was calculated using simple linear least-squares regression formulae (Sokal and Rohlf 1981) using machine readout (x) and standard lengths (y). Line drawings (10, 20, 30, 40, 50, and 60 mm long) placed at 45° angles to perpendicular were used as standards. Two readings on each standard length, before and after rotating the acetate 90°, were taken. The program calculated the mean of each pair of values and produced a calibration curve from known values. Parameter estimates for each calibration curve and goodness-of-fit statistics were automatically displayed after calibration data were entered. R^2 values for these curves were never <0.99.

3) Two readings at 90° to one another were taken for each root sample, and the means of each pair were used to obtain estimates from the calibration curve. The range of root lengths was restricted to ≤ 60 mm. This prevented overlap of root segments and allowed adequate resolution of small diameter roots. Consequently, samples were subdivided, with no single subsample having >60 mm (total) root length. The program totalled the subsamples after the entire sample had been measured.

For the Reference procedure:

1) Dissected root segments were placed parallel on moistened filter paper, then placed on a movable table and roots were viewed with a dissecting microscope equipped with intersecting crosshairs. Root ends were placed at the vertical crosshair and root axes parallel to the horizontal crosshair.

2) A hand crank moved the table, allowing an operator to measure lengths as roots moved past a crosshair in the field of view. Length measurements were displayed on a monitor.

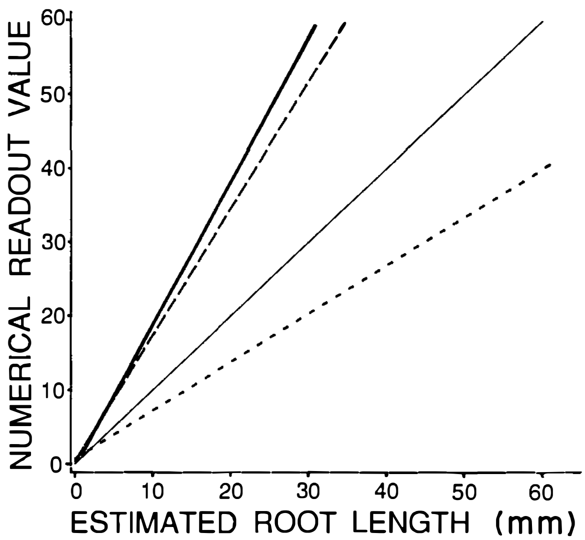

FIG. 1. Examples of calibration curves used in the Right Angle method. One curve for each diameter class is shown (----- 0.00–0.49, — — — 0.50–1.50, ——— 1.51–3.00 mm). A curve with a slope of 1 is shown for comparison (——).

Root samples from three diameter classes were used in the analysis (0.00–0.49, 0.50–1.50, and 1.51–3.00 mm), and line widths of the standards corresponded to midpoints of the diameter ranges. Each root sample was measured using all four methods in the following order: Semirandom, Right Angle, Parallel, and Reference. Root arrangements were identical for the Semirandom and Right Angle methods. Although hardware calibration was not required for the Right Angle method, the camera height and area-meter dials were in identical positions for all three IA methods.

Root samples were from a beech-birch-maple forest (*Fagus grandifolia, Betula alleghaniensis,* and *Acer saccharum*) in the Adirondack Mountains of New York. Live roots were removed from soil cores, cleaned of soil and organic matter, and sorted into three size classes. Samples were stored at 1°C in distilled water.

Precision

The precision of the Right Angle method estimates was evaluated by assessing the repeatability of measurements on individual samples of roots. Six samples from each of the three root diameter classes were measured six times (each iteration involved recalibration as well as remeasurement) and the standard deviation, coefficient of variation, and range were calculated. Precision of the SONY electronic ruler method was determined similarly.

Accuracy

The accuracy of each IA procedure was determined by comparing the root-length estimates derived using the IA with the reference root length measured with

TABLE 1. Comparison of estimates obtained by four root-length measurement methods. Means with different superscript letters in the same column are significantly* different.

Method	Root diameter class (mm) 0.00–0.49	0.50–1.50	1.51–3.00
	Estimated root length (mm)		
Semirandom	52.9[a]	51.1[a]	50.2[a]
Reference	40.6[b]	38.6[b]	32.8[b]
Parallel	38.5[c]	37.1[b]	32.0[b]
Right Angle	37.8[c]	35.8[b]	31.0[b]

* $P \leq .0167$ using Bonferroni's method, where the overall significance level is $P \leq .05$.

TABLE 2. Analysis of percent bias* in two methods. Values followed by different letters in the same column are significantly† different.

Method	Root diameter class (mm) 0.00–0.49	0.50–1.50	1.51–3.00
	Percent bias		
Parallel	−5.2[a]	−3.8[a]	−2.7[a]
Right Angle	−6.8[a]	−7.6[b]	−5.9[a]

* 100 × (root-length estimate − reference length)/reference length.

† $P \leq .0167$ using Bonferroni's method; overall significance level is $P \leq .05$.

the electronic ruler. Percent bias was calculated using the formula

$$\%\text{bias} = \frac{(\text{length estimate} - \text{reference length})}{\text{reference length}} \times 100$$

Speed

The amount of time required to calibrate, arrange, and measure root samples was assessed. Comparisons were made by one operator on four samples from each of three diameter classes for the Parallel, Right Angle, and Reference methods.

Statistical differences in estimating precision and accuracy for each method and for each diameter class were evaluated by one-way ANOVA. Bonferroni's method was used to maintain a constant error rate for comparisons of the three diameter classes (Kirk 1982).

RESULTS

Although the calibration curves used in the Right Angle method were linear ($R^2 > 0.99$), the slopes were not 1 (Fig. 1). The lower the camera was set, the less the slope of the regression line.

Estimates of root length differed significantly among the four methods (Table 1). The Parallel and Right Angle methods gave estimates similar to the Reference values. The Semirandom method overestimated root length by 30, 34, and 52% for small, intermediate, and large root diameter classes, respectively. Thus, no further analyses of data with this method were justified. The Parallel and Right Angle procedures were equally precise and accurate for all size classes, and were in agreement with the Reference method on the intermediate and largest diameter classes. These two IA procedures, however, underestimated the lengths of the smallest diameter class. The Right Angle procedure underestimated root lengths slightly more than the Parallel method, but the differences were generally not significant (Table 2).

The three accurate methods differed in sampling speed. The Right Angle method was most rapid and the Reference method the slowest (Table 3).

Precision was similar between the Reference and Right Angle methods for the smallest diameter size class, but increased in the Reference procedure for the two larger diameter size classes (Table 4). The Parallel method was rejected because of its obvious inefficiency.

DISCUSSION

The Right Angle method for measuring root length was comparable in accuracy and precision to the Parallel and Reference methods (coefficient of variation < 5.0%). Although it slightly underestimated root length

TABLE 3. Sample processing time (in seconds*) for three methods (mean ± SE).

Task	Diameter class (mm)	Method: Parallel	Right Angle	Reference
		Processing time (s)		
Calibration	0.00–0.49	210 ± 21.0	100 ± 2.1	0
	0.50–1.50	170 ± 37.9	90 ± 8.0	0
	1.51–3.00	140 ± 22.4	80 ± 2.5	0
Root arrangement	0.00–0.49	220 ± 32.2	20 ± 1.6	220 ± 32.2
	0.50–1.50	100 ± 18.0	20 ± 3.0	100 ± 18.0
	1.51–3.00	60 ± 23.0	10 ± 1.2	60 ± 23.0
Measurement	0.00–0.49	1 ± 1.2	10 ± 1.2	260 ± 40.2
	0.50–1.50	1 ± 0.5	10 ± 0.9	110 ± 16.7
	1.51–3.00	10 ± 0.2	10 ± 0.2	50 ± 3.3
Total per sample (arrangement + measurement)	0.00–0.49	220	30	480
	0.50–1.50	110	30	220
	1.51–3.00	70	20	110

* Time was rounded to the nearest 10 s.

TABLE 4. Comparison of precision (repeatability) for two methods. Values with different superscript letters in the same column and subsection are significantly* different.

		Root diameter class (mm)		
Measure	Method	0.00–0.49	0.50–1.50	1.51–3.00
SD (mm)	Right Angle	1.1[a]	1.0[a]	0.9[a]
	Reference	1.0[a]	0.3[b]	0.3[b]
CV (%)	Right Angle	2.8[a]	2.5[a]	2.9[a]
	Reference	2.6[a]	0.8[b]	0.9[b]
Range (mm)	Right Angle	2.8[a]	2.6[a]	2.3[a]
	Reference	2.7[a]	0.9[b]	0.7[b]

* $P \leq .0167$ using Bonferroni's method, where overall significance level for each measure is $P \leq .05$.

in the smallest diameter size class, precision was high and the estimates could be corrected during calibration. The Semirandom method was inaccurate, producing estimates with a large and inconsistent bias.

Precision of the Reference method was high in the two largest diameter classes, because roots of these size classes were relatively straight and easily measured. Precision with the smallest roots was lower because of their highly branched nature.

The Right Angle method was faster because camera placement and root orientation had less stringent requirements than other methods. Both IA methods were superior to the tedious and time-consuming Reference method.

The Right Angle method has four advantages over previously described methods (Rowse and Phillips 1974, Richards et al. 1979, Heringa et al. 1980, Voorhees et al. 1980, Barnett et al. 1987). First, it allows a high degree of flexibility in camera height without compromising precision and accuracy. This means that roots with low contrast need not be stained or photographed. Second, estimates are not influenced by root orientation. Third, real time calibration eliminates the need to convert data after collection. Fourth, the length estimates may be accumulated and stored in computer files via a menu-driven program.*

* See ESA Supplementary Publication Service Document No. 8801. For a copy, contact the senior author or order from The Ecological Society of America, 328 East State Street, Ithaca, NY 14850-4318 USA.

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