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Comparison of Reporting Methods for Root Growth Data from Transparent-Interface Measurements

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ABSTRACT

Transparent viewing planes provide a useful and popular means of observing and quantifying root growth. This study was conducted to compare root growth quantification by soil depth and distribution using three tracing methods of reporting root growth: (i) traced root intensity (traced RI, (ii) traced root-length density (traced RLD), and (iii) traced root-length area (traced RLA. These three root-tracing methods were compared with actual RLD and actual Kl. V determinations made from the bulk soil volume at the termination of the study. Creeping bentgrass *{Agrostis stolonifera* L.; syn *A. palustris Huds.*) sod was planted in clear, flexible polyethylene containers slanted at a 20° angle. During a period of =2 mo, root growth by depth was monitored by tracing roots onto clear polyester sheets. None of the reporting methods for traced roots generated root growth measurements equivalent to actual rooting. Traced RLD yielded the best estimates of root quantity at each soil depth, yet, generated poor root distribution estimates. Traced RLA yielded the most accurate estimate of root distribution compared with actual root distribution when data were transformed to a percent basis. Flexible, transparent polyethylene containers used for viewing-plane measurements of root growth quantification and distribution can, when reported by these three methods, differ substantially from actual bulk soil RLD or RLA. Thus, this transparent viewing-plane procedure exhibits the same problems inherent in root measurements from rhizotrons and minirhizotrons.

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TRANSPARENT viewing planes are a nondestructive means of monitoring and quantifying root growth. Utilization of transparent viewing planes occurs in one of three manners: (i) rhizotrons, (ii) minirhizotrons, and (iii) clear-fixed containers. Rhizotrons are underground root observation laboratories that contain a transparent surface placed against soil. Minirhizotrons utilize narrow diameter tubes (usually < 10 cm) inserted into the soil; usually, a small video camera placed down the tube allows observation of the root system. Clear-faced containers offer an alternative to the more expensive rhizotron and minirhizotron systems. Rigid-walled boxes, pots, and tubes have been traditionally used, but recently flexible polyethylene tubes have become availalble (7). The ideal for quantifying root growth for any transparent viewing-plane procedure is to provide an accurate estimate of bulk soil root-length density. Root water and nutrient uptake are best related to RLD and models for uptake use RLD data (15).

Huck and Taylor (6) suggested that rhizotrons allow only anatomical observations and measurement of root extension rates but not accurate measurement of bulk soil root length and density. With the clear-faced container method using slanted (25°), cylindrical Plexiglas tubes (7.6 cm diam. by 7.6 cm), Voorhees (17) found root elongation rates along a viewing plane different from bulk soil elongation rates, and suggested that increased soil bulk density along the glass-soil interface restricted root growth. He also alluded to a possible electrical charge

Abbreviations: RI, root intensity; RLA, root-length area; RLD, root-length density; UV, ultraviolet.

along the Plcxiglas, which may have reduced rooting due to a root electrical charge (11). Attempting to correlate root measurements from minirhizotrons to bulk soil RLD continues to be addressed by research scientists (1, 15, 18). Glinski and Lipiec (5) and Upchurch (15) provide recent reviews on this topic.

Evaluation of the polyethylene, flexible tubing approach with respect to bulk soil vs. transparent viewing-plane root measurements has not been conducted. Lehman and Engelke (7) used the flexible tubing, but did not compare their transparent surface measurements to bulk soil RLD. Also, the flexible tubing differs from previous methods in physical (flexible vs. rigid) and chemical (polyethylene vs. glass, Plexiglas, or acrylic) characteristics. Current literature contains limited information on the correlation between root growth along the angled face of clear-faced containers and actual root growth in bulk soil. This contrasts to multiple investigations of the same issue on rhizotrons or minirhizotrons (1,2,10,15,16,18,19).

A problem in comparing root growth data from any transparent vicwing-plane method to bulk soil rooting is the method chosen for reporting the data. Bulk soil rooting is normally presented in terms of RLD or root weight per unit volume of soil. Rooting data from transparent viewing-plane procedures may be reported as traced root length per unit of traced area, traced root length per unit volume of soil, traced root length per surface area of soil surface, or root intersections at different soil depths (15). An important consideration is which reporting procedure from transparent viewing-plane measurements best correlates to actual bulk soil rooting, both quantitatively at each depth and with respect to distribution.

Thus, the purpose of this study was to compare RLD of the bulk soil to transparent interface measurement data using the flexible, polyethylene tubing as a clear-faced container. Several different methods for reporting the transparent interface data were used, to determine which reporting method best correlates with bulk soil RLD for root quantity at a given depth and for overall root distribution.

MATERIALS AND METHODS

'Penncross' creeping bentgrass was grown in flexible, transparent, polyethylene containers (McMaster-Carr Supply Co., Chicago, IL). Cylindrical tubes (16.5-cm diam. by 60 cm; 0.076-mm wall thickness) contained a commonly used medium for golf course greens of 85% sand (889r of particles of 0.25-1.0-mm diam. and 11% <0.25-mm diam.) and 15% Michigan peat (by volume). Soil tests indicated soil pH, P, and K were below Georgia Extension Service recommendations. Calcium hydroxide was added to increase pH to 6.5. Phosphorus and K were increased to 42.6 kg P ha^{-1} (medium) and 97.6 kg K ha ' (high) with KH_2PO_4 and KCl. Nitrogen was initially applied as a solution of NH_4NO_3 (24.4 kg N ha^{-1}).

After mixing the chemical and physical amendments, one end of the tube was folded, sealed with staples, and filled with the amended soil. One-kilogram increments of soil were successively packed into the containers. Packing continued until tubes were filled and wrinkles in the plastic were removed. All 12 tubes were of equal volume and received equal volumes of soil. The bulk density was 1.54 Mg m \ After packing, tubes were set on a plywood A-frame structure at 20° from vertical. To promote soil settling, tubes were saturated with water and remaining wrinkles removed by tamping. Soil heights varied (± 2 cm) and were adjusted to equivalent levels.

On 15 Feb. 1987, Penncross creeping bentgrass plugs (17.8 cm diam. by 10 cm) were cut from an experimental golf green at the University of Georgia Turfgrass Demonstration Plots in Athens, GA. Soil was washed from the plugs, and roots were removed to just below the plant crown. Plugs were fit into the soil containers and watered. To exclude light from the roots, each tube was wrapped in two layers of 0.1-mm black polyethylene plastic. A white plastic sheet was placed over the black, to reduce thermal effects.

Throughout the growing period (15 Feb.-26 May 1987), greenhouse temperatures was maintained at 24 ± 5 °C. On two clear, sunny days, soil temperatures were measured at several depths by inserting a thermometer through the side of extra tubes to different insertion lengths. Soil temperatures at all depths and insertion lengths corresponded with air temperatures within ±2 °C. Fluorescent lights at 30 cm above the turfgrass surface supplemented natural lighting from 0600 to 1800 h.

The turfgrass was cut daily at a height of 5.0 mm. Water was applied as needed; all applications were sufficient to saturate a few centimeters below the deepest-appearing roots of the deepest-rooted tube. Actual application rates approximated 2.6 cm of water every other day. In addition to the initial NH_4NO_3 application, N was applied monthly as urea, with a total of 85 kg N ha^{-1} added throughout the study. To maintain contact between the turf and plastic at the top of each tube, all tubes received three light sand topdressings over the period of the study.

Root measurements began on 29 March. Trans-Art Trans-Stay Clear Polyester sheets (Transilwrap Company of Atlanta, Atlanta, GA) were taped to each tube. Roots within a 10-cm-wide band extending the length of the tube were traced onto the sheets on the lower sidcwall of the slanted tubes. Since Cooper et al. (3) reported that bentgrass roots fluoresce with exposure to UV light, thereby increasing traccability, tracings were made at night under UV lighting (320-400 nm) generated by a Model B-100A/R Black-Ray Ultra-Violet lamp (UVP, San Gabriel, CA). Tracings were made once a week, using Stacdler Lumicolor permanent marking pens (Stacdler, Niirnberg, Germany). Different colored markers distinguished dates. Root lengths were determined by measuring the traced roots at 5-cm depth intervals with a LAS1CO Model 71A linear measuring probe (Los Angeles Scientific Instrument Co., Los Angeles, CA).

Bulk Soil Root Procedures

On 26 May 1987, verdure (consisting of leaves, stems, crowns, and stolons) was separated from the roots by cutting through the plastic immediately below crown level. Tubes were cut into 10 5-cm segments (50 cm total depth), cut parallel to the original turfgrass surface. Roots were collected by hand-sifting segments through a 1.0-mm screen and then were stored in a freezer until 3 Aug. 1987. Root length was determined using the root intercept method and Newman's equation, and root weights for each zone were made (8). Due to the large quantity of roots >30 cm, four subsamples were randomly obtained from the total root mass in each soil zone and used to determine root lengths. After drying (70 °C for 24 h), lengths of the bulk samples were calculated from length and weight of the subsamples and weight of all roots in a soil zone.

These bulk soil values are reported as actual-RLD (root length per unit volume of soil; cm cm^{-1}) and actual-RLA (root length per area of sod directly above and to the uppcr-vicwing-planc side of the soil zone; cm cm^{-2}). The values of actual RLD and actual RLA were based on an average of the 12 tubes, where all soil in the tubes was sieved for roots by soil depth as described previously.

Traced Root Procedures

Root data from tracing the transparent surface length in each soil zone were presented by three different procedures, in order to compare with actual RLD and actual RLA data. The three reporting procedures were: (i) traced root intensity (traced-RI) equaled the length of traced roots per unit of tracing surface area; (ii) traced root length density (traced RLD) equaled the traced nx)t length per volume of soil where soil volume equaled the tracing area x the approximate depth at which roots could be seen (3 mm); and (iii) traced root length per area of sod (traced RLA) equaled the traced root length per area of sod (directly above and to the up-vicwing-planc side of the tracing depth).

Actual RLD and traced RLD data were subjected to paired comparison at each soil depth, using the RLD values generated by each procedure for the 12 replications. A similar analysis was performed on the actual vs. traced RLA data. This analysis provided information on root quantification (Table 1) at each depth.

To compare the root reporting methods for ability to describe root distribution (i.e., relative rooting), all data were transformed to percentage roots occurring at each depth (Fig. 1). Root distribution over the complete profile was described by determining the best curve that fit the data by nonlinear regression. Data from each procedure were compared with other procedures based on a significant /•'-test and treatment (procedure) mean separation by LSD (0.05). In addition, linear regression analysis was used as another approach to describing root distribution using raw data.

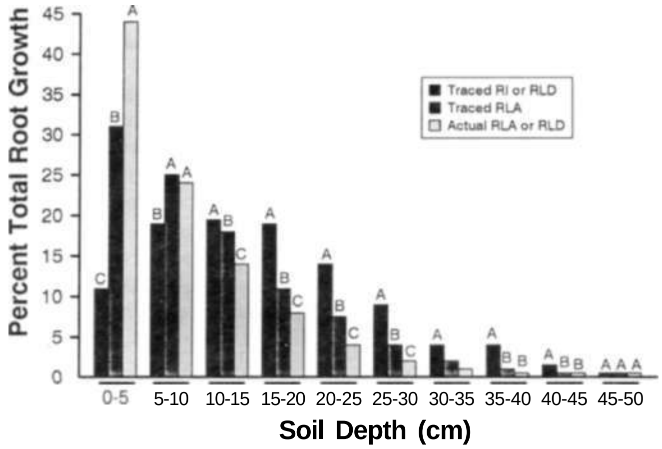

Fig. 1. Comparison between actual and traced methods for describing root distribution using nonlinear regression and with the data based on percent of total roots at a particular soil depth. Traced RI = traced root intensity, Traced RLD = traced root-length density, Traced RLA = traced root-length area. Values with different letters on the top of bars signify statistical difference at that depth using LSD at the 0.05 level.

RESULTS AND DISCUSSION

Root Quantification

Bulk soil root quantification was determined in three ways: actual RLD, root weights, and actual RLA (Table 1). Since root length is the preferred way to report rooting data for nutrient and water uptake studies, we concentrated on actual RLD and actual RLA in comparison with the traced reporting methods. The accuracy of the traced RI method could not be determined for root quantification, since the method measures two-dimensional rooting and the actual sample for RLD is three dimensional. Evaluation of this method is limited to root distribution, which will be discussed later.

To eliminate the sample dimension problem that occurs with the two dimensional traced RI method, several researchers (10,12,13,14,16,17) quantify rooting as traced RLD. This technique utilizes rooting density by multiplying traced RI by an estimated depth of view for traceable roots. Commonly used depths include 1 to 2 mm (17), 2 mm (11,12,14), and 3 mm (10), which was used in our study.

In this study, actual RLD differed from traced RLD (Table 1) at most depths. At soil depths between 10 to 45 cm, traced RLD values ranged from 25 to 89% more than actual RLD. Traced RLD tended to increase as depth increased relative to actual RLD. Apparently, traced RLD

Table 1. Comparison of various methods of reporting root growth determined from a transparent interface method with actual bulk soil rooting (averaged across 12 replicates) at the termination of the experiment.

Depth	Weight†‡	RLD comparisons§		RLA comparison¶		Traced RI#	CV††
		Actual‡	Traced	Actual‡	Traced		
cm	mg cm⁻²	cm cm⁻³		cm cm⁻²		cm cm⁻²	%
0–5	3.106	22.60a‡‡	7.09b	113.0a	13.3b	106	15
5–10	1.078	12.61a	11.58a	63.0a	10.8b	174	18
10–15	0.538	7.46b	12.30a	37.3a	7.7b	185	25
15–20	0.312	4.60b	11.06a	23.0a	5.2b	166	24
20–25	0.197	2.47b	8.90a	12.4a	3.3b	134	30
25–30	0.079	0.95b	5.86a	4.8a	1.8b	88	35
30–35	0.042	0.45b	2.81a	2.3a	0.8b	42	34
35–40	0.019	0.16b	1.17a	0.8a	0.3b	18	40
40–45	0.011	0.08b	0.71a	0.4a	0.1a	13	38
45–50	0.001	0.01a	0.04a	0.1a	0.0a	1	40

† Milligrams of dry root per square centimeter of sod surface.
‡ A measurement of actual bulk soil rooting.
§ Actual RLD = actual root-length density in centimeters of root (obtained by line intersect method and Newman's equation) per cubic centimeter of soil; determined from root lengths obtained in each soil zone. Traced RLD = centimeters of traced root per cubic centimeter of soil, assuming that only roots within 3 mm of the viewing plane are traceable.
¶ Actual RLA = actual root-length area in centimeters of root (obtained by line intersect method and Newman's equation) per square centimeter of sod surface; determined from root lengths obtained in each soil zone. Traced RLA = centimeters of traced root per square centimeter of sod.
# Trace RI = traced root intensity in centimeters of traced root per square centimeter of tracing area.
†† Coefficient of variation of actual RLD data among the 12 tubes (replicates) at each soil depth.
‡‡ Within rows, means followed by different letters differ at the 0.05 level (*F*-test).

becomes increasingly more misleading as depth increases. This may reflect the influence of angle (20°) of the angled viewing plane, which confines roots to a smaller soil volume with depth. Also, the higher traced RLD values could be due to enhanced rooting caused by the plastic interface (12), or an underestimate of the viewing-plane depth (3 mm).

Using the traced RLD method in rhizotrons (vertical, acrylic plastic viewing plane), Taylor and Klcpper (14) obtained traced RLD by multiplying traced root lengths (traced Rl) by a depth of 2 mm. Comparing these values with actual density (obtained from root weights, assuming roots were 95% water and 0.04 cm diam.), they found interface effects caused errors up to 50%.

Probably the least-used way to measure root quantity involves reporting traced root length per unit ground area (traced RLA). Several early root investigators (4,9), not using rhizotrons, reported grass root length per unit ground area. In rhizotrons, Taylor et al. (13) gave brief mention of expressing root growth per unit ground area.

Our traced RLA values drastically underestimated actual rooting per area of sod (actual RLA) (Table 1). The greatest differences existed between 0- to 25-cm depths, where traced RLA values were only 11.8 to 26.6% of actual RLA. The cause of low traced RLA values remains unclear. Possibly, traced RLA differed from actual RLA for a combination of inherent reasons: off-and-on appearance of roots may affect results; roots do not necessarily grow vertically; and concentration of roots along the interface may cause competition and reduce growth.

Thus, traced RLD and traced RLA exhibited substantial errors for quantifying roots at each depth when compared with actual RLD and actual RLA, respectively. Traced RLD, however, was more accurate than traced RLA for root quantification. Apparently, the transparent, flexible, polyethylene tubing offers no advantage over rhizotron or minirhizotron methods for root quantification by soil depth.

Root Distribution

Linear Regression

One approach for comparing traced rooting methods vs. actual bulk soil rooting is linear regression over the whole rooting depth (Table 2). Even with a high coefficient of determination (r^2), however, caution must be exercised with linear regression. This can be illustrated by comparison of actual RLD vs. root weights (Tables 1 and 2). Very high values for root weights and actual RLD in the surface 10 cm skew the linear regression toward greater accuracy as root weights increase. For example, at 3.11 mg cm^{-2} root weight, the estimated RLD is 24.26 cm cm^{-3} (actual RLD is 22.60 cm cm-'), while at a root weight of 0.02 mg cm^{-2} the estimated RLD is 1.27 cm cm^{-3} (actual RLD is 0.16 cm cm^{-3}). This illustrated that higher numbers for root weights and actual RLD within the upper root zone of a plant predisposes the linear regression analysis to be less accurate in the lower root zone. Also, not all roots were of the same degree of fineness at each soil depth. Thus, the length of roots per unit weight of roots may differ substantially with depth.

The regression equation for actual RLD vs. actual RLA would be expected to show an excellent linear relationship since both values are calculated from the root length found within a soil zone (Table 2). But, one set is reported on a volume basis and the other on an area basis.

Table 2. Linear regression for comparison of actual bulk soil rooting vs. traced-root reporting methods.

Regression variables				
Dependent (Y)	Independent (X)†	Linear regression equation	r^2	Prob > F
Actual RLD	Traced RLD	$Y = 0.253 + 0.797X$	0.26	0.129
	Traced RLA	$Y = -1.252 + 1.48X$	0.92	0.0001
	Traced RI	$Y = 0.195 + 0.053X$	0.26	0.129
Actual RLA	Traced RLA	$Y = -6.23 + 7.38X$	0.92	0.0001
	Traced RLD	$Y = 1.19 + 3.99X$	0.26	0.129
	Traced RI	$Y = 1.01 + 0.27X$	0.26	0.129
Actual RLD	Weight	$Y = 1.13 + 7.45X$	0.95	0.001
	Actual RLA	$Y = -0.006 + 0.200X$	1.00	0.001

† Traced RLD = traced root-length density (cm cm^{-3}); traced RLA = traced root-length area (cm cm^{-2}); traced RI = traced root intensity (cm cm^{-2}).

Traced RLD exhibited a low coefficient of determination for the linear regression with actual RLD (Table 2). Traced Rl had the same r^2 as traced RLD because traced RLD is calculated from traced Rl; however, their linear regression lines differ due to different numerical values for each tracing method. While traced RLA demonstrated the greatest linear relationship with actual RLD, at traced RLA values below 0.85 cm cm^{-2}, actual RLD is predicted to be zero. This represents all actual rooting below 30 cm. As discussed previously, the large numerical values of actual RLD and traced RLA in the upper half of the root zone skews linear regression toward this region.

One possible approach for estimating actual-RLD of a plant from a traced-root method exhibiting a high r^2 for linear regression over the whole rootzone would be to develop two sets of regression lines: one for the surface and another for the deeper soil profile. When this was done for actual RLD vs. traced-RLA, the regression equations were $y = -5.38 + 1.90x$ ($r^2 = 0.92$) and $y = -0.001 + 0.534x$ ($r^2 = 0.99$) for soil zones 0 to 25 cm and 25 to 50 cm, respectively. These proved to be more accurate than the single equation when various values of traced RLA were used; with the first equation using from 3.3 to 13.3 cm cm^{-2} traced RLA and the second for a range of 0 to 3.2 cm cm^{-2}. As with any traced-rooting method to estimate actual RLD, the regression lines must be developed by calibration for each crop and soil (1).

Since actual RLD and actual RLA differ only in calculation method, traced RLA should relate to both in the same manner. This was the case as demonstrated by the same r^2 (Table 2).

Therefore, when linear regression was used to describe root distribution by traced-rooting methods vs. actual RLD and actual RLA, only when data was reported as traced RLA did a good linear relationship exist. Low r^2 values were apparent for traced RLD and traced Rl compared with actual bulk soil rooting.

Curve Fitting

A second approach to comparing actual bulk soil root distribution to distribution by traced-root methods is to compare the best root distribution curve for each method. To evaluate the ability of the various tracing methods to predict actual root distribution, growth was converted to a percentage of roots occurring at each depth (Fig. 1). Since traced RI and traced RLD originate from the same data base, and differ only by a constant conversion factor (3 mm = estimated viewing-plane depth), distribution for both techniques is identical on a percentage basis. Also, actual RLD and actual RLA have the same percentage distribution since they are derived from a com-

Root distribution curves varied among methods of presenting the data (Fig. 1). Root distribution curves were: for actual RLD and actual RLA, $y = -4.97 + 51.1/x$ ($r^2 = 0.94$); for traced RLD and traced Rl, $y = 1.01 + 14.2x - 3.17x^2 + 0.18x^3$ ($r = 0.88$); and for traced-RLA, $y = -0.74 + 36.68/x$ ($r^2 = 0.69$), where y = percent root growth and x = soil depth in cm. The curve for root weights was $y = -8.65 + 63.7/x$ (not presented in Fig. 1). While slopes differed, curves for root weight, actual RLD, actual RLA, and traced RLA had similar reciprocal shapes. The reciprocal distribution is reflective of rooting patterns observed in field situations. In contrast, root distribution by traced RLD or traced RI generated a quadratic shape.

Relative to actual RLD, traced Kl, and traced RLD values underestimated actual root distribution near the surface 10 cm and overestimated rooting at deeper depths. Several researchers (3,4,13,17) have used root extension rate and traced RI to evaluate root activity. Researchers (13,14) have reported good correlation between rooting along the interface and bulk soil rooting in vertical viewing-planes of rhizotrons. No data were found from angled viewing-planes in rhizotrons or clear-faced containers.

The traced RLA method, compared with actual RLA, also underestimated root distribution at shallow depths (5 cm) and overestimated rooting at deeper depths. However, traced RLA provided a reciprocal curve with values that better represent actual root distribution than traced RI or traced RLD, and, therefore, appears to be the most accurate method of evaluating bulk soil root distribution.

In conclusion, tracing roots on transparent viewing-planes is valuable when making qualitative observations such as root morphology and/or determining root extension rate. However, as suggested by Huck and Taylor (6), and shown in this study, angled transparent viewing-planes can be misleading for predicting bulk soil root length, root length density, and root distribution. None of the tracing methods (traced RI, traced RLD, and traced RLA) accurately depict bulk soil root growth as determined by actual RLD at the termination of the study. The traced RLD method provided by the best tracing procedure for evaluating actual root quantities at any particular depth. However, when evaluating root distribution, traced RLD may be very misleading and traced RLA provided a more accurate estimate. The transparent, flexible, polyethylene tubing did not eliminate any of the difficulties encountered by other transparent viewing-plane procedures used to measure plant rooting.

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