THE INFLUENCE OF VIEWING-PLANE ANGLE ON ROOT GROWTH QUANTIFICATION USING THE TRANSPARENT-INTERFACE METHOD[1]

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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 determine the effect of angled viewing-planes on root growth quantification and distribution using three methods of reporting root growth (root intensity, root length density and root length per area of ground). Creeping bentgrass (Agrostis palustris Huds.) sod was planted in clear polyethylene containers slanted at 20° angles. During a two month period, root growth was monitored by tracing roots onto clear polyester sheets. At the end of the growing period, actual root growth was measured and compared to traced root growth. None of the tracing methods generated root growth measurements equivalent to actual rooting. Root length density estimates yielded the best quantification of root quantity, yet, generated poor distribution estimates. The root length per unit ground area yielded the most accurate estimates of root distribution. This study indicates angled viewing-planes influence measurements of root growth quantification and distribution.

Plant researchers have long recognized the importance of root growth, while experiencing difficulty in monitoring growth in a non-destructive manner. Transparent viewing-planes offer a promising, non-destructive means of monitoring and quantifying root growth. Typically, utilization of transparent viewing-planes occurs in one of three manners: 1) glass-face boxes, 2) rhizotrons, and 3) minirhizotrons. Glass-face boxes consist of portable boxes, pots, or tubes which contain a transparent surface. Rhizotrons are underground root observation laboratories which contain a transparent surface placed against soil. Minirhizotrons utilize narrow diameter tubes (usually less than 10 cm) inserted into the soil; a small video camera placed down the tube allows observation of the root system. Although all three methods provide a means of monitoring root growth, they differ dramatically in design. Perhaps, the greatest variable between, and within, the various methods is viewing-plane angle.

Rhizotrons incorporate vertical and/or angled viewing-planes. In the past, rhizotrons tended to use vertical viewing-planes [Griffith, N.S.W. Australia (6); University of Guelph, Ontario, Canada (8); Muscle Shoals, Alabama (22); Auburn, Alabama (27); Ames, Iowa (24); College Station, Texas (1)]. The designer of the first rhizotron, W. S. Rogers, considered vertical viewing-planes crucial for success since they helped overcome the tendency for finer soil particles to accumulate at the interface and obscure root growth (19). Although vertical orientations once predominated, many rhizotrons have incorporated angled viewing-planes: 10° at Auburn, Alabama (27); 15° at Texas A&M (4); 20° at Ohio State University, Columbus, Ohio (10); 12.5° at University of Georgia, Athens, Georgia (12); 23°at University of Nebraska (21); 10° at a temporary pit-rhizotron on Kansas (7). Glass-faced boxes and minirhizotrons also utilize a plethora of viewing-plane angles, ranging from vertical to 45°(31 29 20 2 30).

The main reason for using angled viewing-planes is to increase the amount of observable roots. Angled planes may allow observation of nearly half the root system (9). Although angled planes provide the advantage of increasing observable roots, Huck and Taylor (9) suggest they allow only anatomical observations and measurements of root extension rates, not measurements of root length and density.

Current literature contains limited information on the correlation between root growth along an angled viewing-plane and actual root growth. Although Bohm (2) found poor correlation between actual and traced rooting using vertically inserted minirhizotrons, other researchers (31 29 20) have reported good correlations using vertically inserted minirhizotrons, especially at depths below 20 cm. With the glass box method, Voorhees (30), using slanted (25°), cylindrical plexi-glass tubes (7.6 cm diam X 7.6 cm), found root elongation rates along a viewing-plane different from bulk soil elongation rates. Voorhees (30) suggested increased soil bulk density along the glass-soil interface restricted root growth. He also eluded to a possible electrical charge along the plexi-glass, which may have reduced rooting due to a root electrical charge (23). As of yet, no information exists which correlates rooting along an angled viewing-plane and rooting in the soil. The purpose of this paper was to present data and discussion which evaluate the effect of viewing-plane angle on apparent root growth (quantity and distribution) using the transparent interface method, and illustrate the relationships between various reporting methods. Since roots within mini-rhizotrons can grow around the angled viewing-plane, thereby altering possible angle effects, discussion will focus on glass-face box and rhizotron systems which contain the roots, and more likely reveal an angle effect.

MATERIALS AND METHODS

'Penncross' creeping bentgrass was grown in transparent polyethylene containers (McMaster-Carr Supply Company, Chicago, IL) to determine the influence of viewing-plane angle on root growth quantification and distribution. Cylindrical tubes (16.5 cm diam x 60 cm) contained a commonly used medium for golf course greens of 85% sand (locally referred to as brown sand; 88%=0.25-1.0 mm, 11%<0.25) 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 $P\cdot ha^{-1}$ (medium) and 97.6 kg $K\cdot ha^{-1}$ (high) with KH_2PO_4 and KCl. Nitrogen was initially applied as NH_4NO_3 (24.4 kg $N\cdot ha^{-1}$).

After mixing amendments into the soil, one end of the tube was folded and sealed with staples then filled with the amended soil. One kilogram (approximately) allotments of soil were successively packed into the containers. Packing continued until tubes were filled and all wrinkles in the plastic were removed. All tubes were of equal volume and received equal volumes of soil. The approximate bulk density was 1.54 $g\cdot cm^{-1}$. After packing, tubes were set on a plywood A-frame structure at 20° from vertical. To promote soil settling, tubes were saturated with water. Further wrinkles were 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 x 10 cm) were cut from an experimental golf green at the University of Georgia Turfgrass Demonstration Plots in Athens, Georgia. Soil was washed from the plugs, and the majority of roots were removed. 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 plastic. A layer of plastic was also wrapped around the front of all the tubes.

Throughout the growing period (15 Feb.-26 Mar. 1987), greenhouse temperature was maintained at 23.9 ± 5°C. On several occasions temperature readings indicated night-time lows of 14.4°C and day-time highs of 39.4°C. On two occasions, soil temperatures were measured at several depths by inserting a thermometer through the side of extra tubes. Soil temperatures at all depths corresponded with air temperatures. Fluorescent lights at 30 cm above the turfgrass surface supplemented natural lighting from 0600 to 1800 h.

The turfgrass was cut daily at heights between 3.1 and 6.3 mm. Water was applied as needed; all applications were sufficient to saturate a few cm 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, all tubes received three light sand topdressings.

Root measurements began on 29 March. Trans-Art Trans-Stay Clear Polyester sheets (Transilwrap Company of Atlanta, Inc., Atlanta, GA) were taped to the back of each tube. Roots within a 10 cm wide band extending the length of the tube were traced onto the sheets. Since, Cooper et al. (3) reported bentgrass roots fluoresce with exposure to ultraviolet light (UV), thereby increasing traceability, tracings occurred at night under UV lighting (320-400 nm) generated by a Model B-100A/R Black-Ray Ultra-Violet Lamp (UVP, Inc., San Gabriel, CA). Tracings were made once a week, using Staedtler Lumicolor Permanent marking pens (Staedler, Nurnberg, West Germany). Different colored markers distinguished dates. Root lengths were determined by measuring the traced roots at 5 cm depth-intervals with a LASICO Model 71A Linear Measuring Probe (Los Angeles Scientific Instrument Company, Los Angeles, CA).

On 26 May 1987, verdure was separated from the roots by cutting through the plastic immediately below crown level. Tubes were divided into ten 5 cm segments (50 cm total depth) which were cut parallel to the original turfgrass surface. Roots were collected by hand sifting segments through an 1.0 mm screen (18 mesh) and then stored under refrigeration until 3 Aug. 1987. Root length was determined using the root intercept method and Newman's equation (13). Due to the large quantity of roots above 30 cm, four sub-samples were used to calculate root lengths. After drying samples (70°C for 24 h), length of the bulk samples were interpolated from length and weight of the sub-samples.

Root growth was evaluated by several methods: 1) root intensity (RI) equaled the length of traced roots per unit of tracing area; 2) traced root length density (31) (traced RLD) equalled the traced root length per volume of soil where soil volume equaled the tracing area x the depth at which roots could be seen (3 mm) (31); 3) traced root length per area of sod (traced RLA) equaled the traced root length per area of sod directly above and to the up-viewing-plane side of the tracing depth. At the end of the study, root weight and length (using Newman's technique as described above) were measured. These values were considered to represent 'actual' RLD and RLA, and were used to evaluate the accuracy of tracing techniques ('traced' RI, RLD, and RLA). The different methods were evaluated for their effectiveness of measuring root quantity (density and length) and distribution.

RESULTS AND DISCUSSION

Traced rooting did not quantitatively represent actual rooting (Appendix B). The accuracy of the first tracing method (RI) cannot be determined since the method measures two dimensional rooting and the actual sample is three dimensional. Evaluation of this method is limited to root distribution which will be discussed later.

To eliminate sample dimension problems that occur with the traced RI method, several researchers (20 24 25 27 28 29 30) quantify rooting as RLD. This technique utilizes rooting density by multiplying traced RI by the depth of traceable roots. Commonly used depths include 1-2 mm (30), 2 mm (24 25 26 27 28) and 3 mm (20).

In this study, actual RLD appeared to differ from traced RLD (Appendix B). At depths below 10 cm, traced RLD values averaged 69% more than actual RLD, while ranging from 25 to 89% more than actual RLD. Traced RLD tended to increase as depth increased, relative to actual RLD. Apparently, traced RLD becomes increasingly misleading as depth increases. This probably reflects the confining nature of angled viewing-planes. Also, the large traced RLD values could be due to enhanced rooting caused by the plastic interface (24), and/or an underestimate of the viewing-plane depth (3 mm). At the deepest depths, a discrepancy appears: traced RLD tend to be more representative of actual RLD. This was probably caused by the small quantity of roots at the deepest depths and does not represent a decrease in the confining effect of the angled interface--the small size and quantity of roots at the deep depths are easy to trace, but difficult to find when sifting.

Using the traced RLD method in rhizotrons (vertical, acrylic plastic viewing-plane), Taylor and Klepper (25) obtained traced RLD by multiplying traced root lengths (traced RI) by a depth of 2 mm. Comparing these values to 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%.

The last, and probably least used manner of measuring root quantity involves reporting traced root length per unit ground area (traced RLA). Several early, non-rhizotron, root investigators (5 14 15 16) reported grass root length per unit ground area. In rhizotrons, Taylor et al. (27) and Dipaola et al. (4) give brief mention of expressing root growth per unit ground area.

Our traced RLA values drastically underestimate actual rooting per area of sod (Appendix B). The greatest differences exist at shallow depths. The cause of low traced RLA values remains unclear. Possibly, traced RLA differed from actual RLA for a combination of inherent reasons: length of roots between off-and-on appearance was not measured; roots do not grow vertically; concentration of roots along the interface may cause competition and reduce growth; and the turf near the edges of the container appeared thinner than other turf. Although the traced RLA values poorly represent actual root growth quantities, they provide value when evaluating root distribution.

All the tracing methods quantitatively differed from actual rooting; nevertheless, tracing methods may represent root distribution (relative rooting). To evaluate the ability of the various tracing methods, in conjunction with the transparent, angled viewing-plane, to predict actual root distribution, growth was converted to a percentage of roots occurring at each depth (Fig 1).

Since traced RI and RLD originate from the same source, and differ only by a constant conversion factor (3 mm =estimated viewing-plane depth), distribution for both techniques is identical. Results suggest traced RI and RLD values underestimated root distribution near the surface 10 cm and overestimated rooting at deeper depths. Several researchers (1 3 5 7 11 17 21 27 30) have used root extension rate and RI to evaluate root activity. Researchers (1 25 27) have found good correlation between rooting along the interface and bulk soil rooting. However, all this data originated from vertical viewing planes. No data was found from angled viewing-planes.

Similar to the traced RI and RLD methods, the traced RLA method underestimated root distribution at shallow depths (5 cm) and overestimated rooting at deeper depths. However, the latter provided values which better represent actual root distribution, and therefore appear to be the most accurate method of evaluating root distribution. This becomes especially evident when viewing shapes of root distribution curves yielded by the different methods (Fig 2).

Comparison of the three quantifying methods (traced RI, RLD, and RLA) methods showed tracing methods do not accurately depict actual rooting since slopes for all curves differ. Although slopes vary, several of the curves had similar shapes. Root distribution by actual weight, and actual RLD/RLA produced reciprocal shapes (r=0.97, 0.90, respectively). The traced RLA also produced a reciprocal shape (0.83). Root distribution by traced RI/RLD generated quadratic shapes (0.94). This indicates the traced RLA method was the most accurate tracing method for reporting root distribution.

Overall, the general increase in traced rooting as depth increased corresponds with the advantages and disadvantages of angled viewing-planes. The angle allows observation of low density root systems by increasing the apparent density. As depth, or angle, increase, the roots become more likely to intersect the viewing-plane (Fig 3). This effect may limit the use of angled viewing planes in studies designed to determine root length, density and distribution. The effect could be especially pronounced in rhizotrons utilizing deep rooted species, which extend 3-5 times the depth of the tubes in this study.

Tracing roots on angled viewing-planes is valuable when making qualitative observations such as of root morphology and/or determining root extension rates. However, as suggested by Huck and Taylor (9), and shown in this study, angled viewing-planes are probably inappropriate for predicting root length, density and root distribution. None of the methods (traced RL, RLD, and RLA) accurately depict actual root growth. The traced RLD method provides the best manner of evaluating actual root quantities at any particular depth. However, when evaluating root distribution the technique may be very misleading. The best method of evaluating root distribution was the traced RLA method.

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Figure 1. Comparison between actual and traced root distribution (values with different letters, on the top of bars, signify statistical difference at that depth using LSD at 5% level).

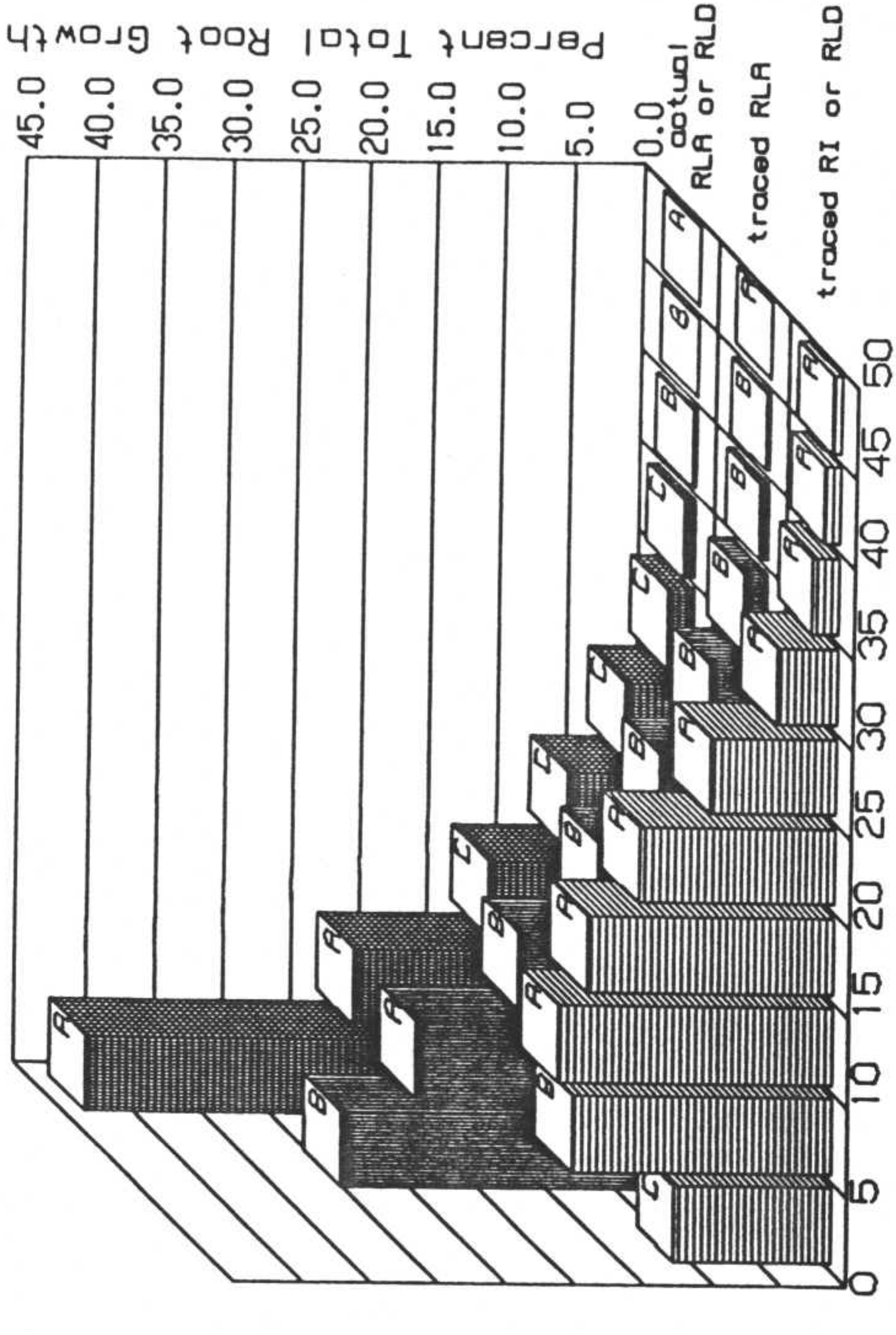

Percent Total Root Growth
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
actual RLA or RLD
traced RLA
traced RI or RLD
Soil Depth (cm)
0
5
10
15
20
25
30
35
40
45
50
A
B
C

Figure 2. Root distribution curves for various quantifying methods: weight, actual RLD or RLA, traced RLD or RI, and traced RLA.

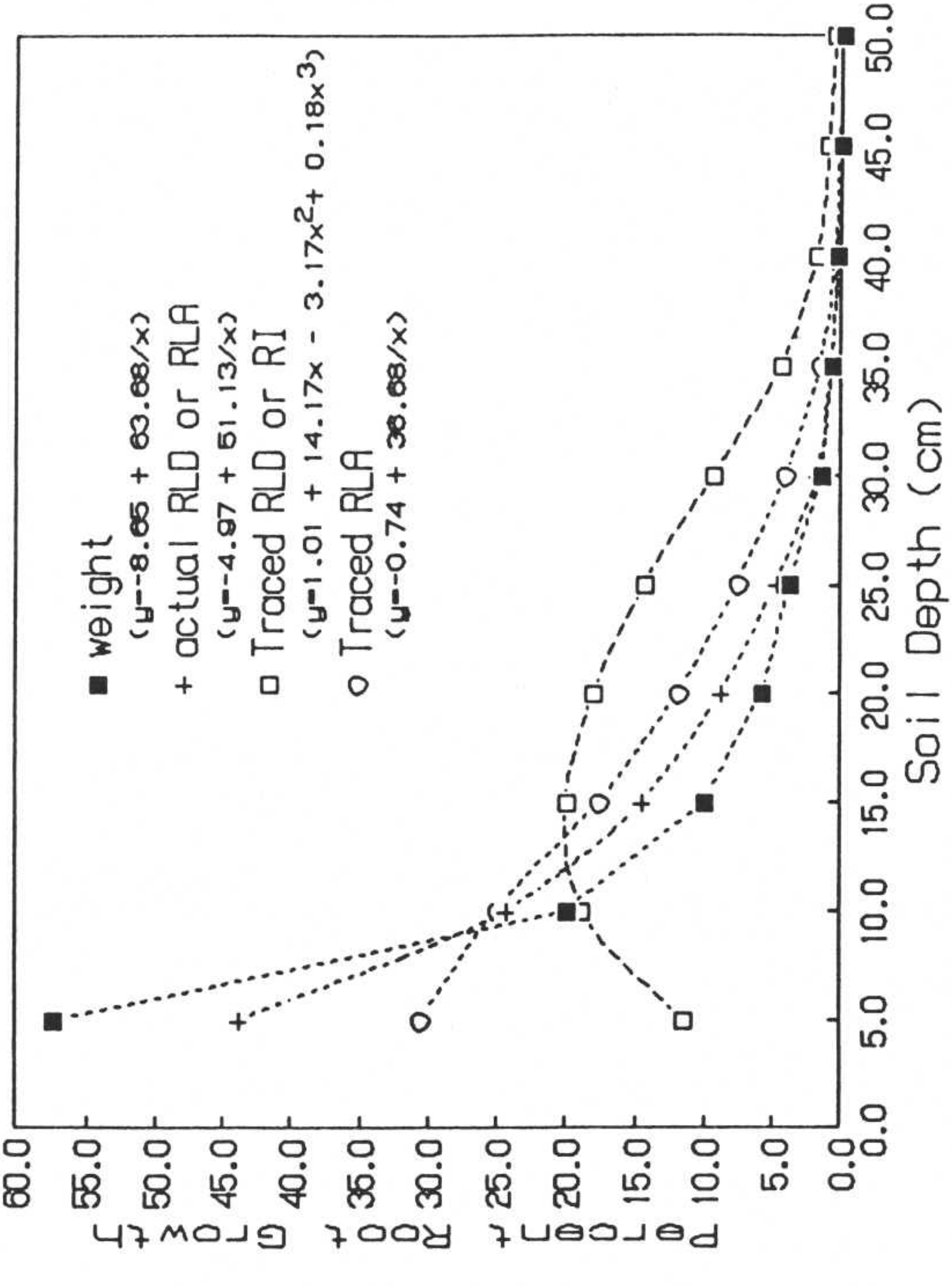
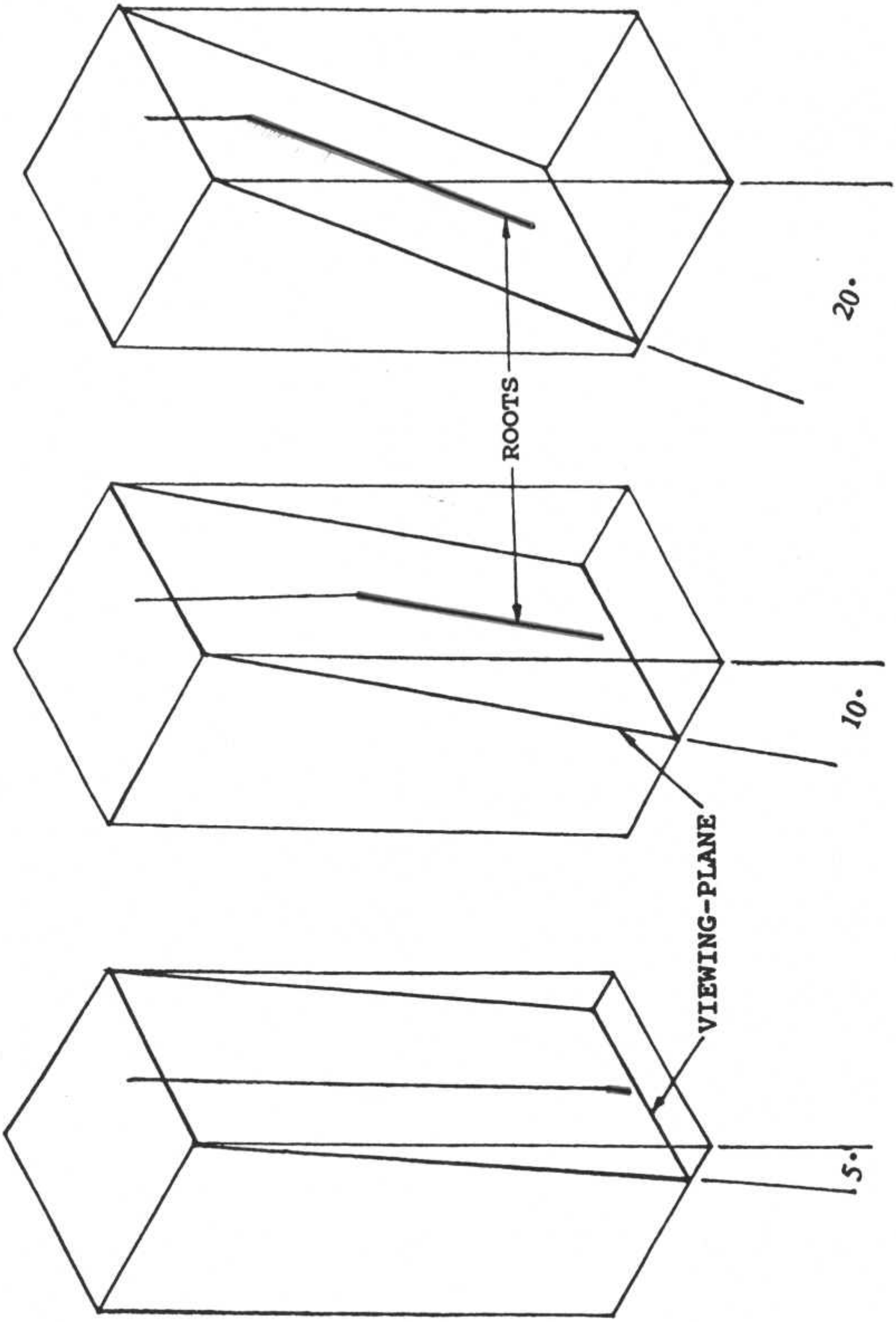

■ weight
(y=-8.65 + 63.68/x)
+ actual RLD or RLA
(y=-4.97 + 51.13/x)
□ Traced RLD or RI
(y=1.01 + 14.17x - 3.17x^2+ 0.18x^3)
○ Traced RLA
(y=-0.74 + 38.68/x)
Percent Root Growth
60.0
55.0
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Soil Depth (cm)
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

Figure 3. Illustration of viewing-plane angle effect on apparent root growth.


ROOTS
VIEWING-PLANE
20°
10°
5°