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Hardware and software efficacy in assessment of fine root diameter distributions

Richard W. Zobel *

USDA-ARS-AFSRC, 1224 Airport Road, Beaver, WV 25813-9423, United States

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ABSTRACT

Fine roots constitute the majority of root system surface area and thus most of the nutrient and water absorption surface. Fine roots are, however, the least understood of all plant roots. A sensitivity analysis of several software programs capable of providing root diameter distribution analyses was undertaken to determine if this software was capable of discriminating 10% changes in diameters of roots in the 0.05–0.2 mm diameter range. Digital images produced by drawing discrete lines, by scanning wires of various diameters, and by scanning roots from several legume species were analyzed and compared. None of the three packages were able to adequately analyze these images. Each introduced artifacts into the data that were severe enough to confound interpretation of the resulting diameter class length histograms at resolutions from 24 to 400 pixels (px) mm^{-1}, and root diameters from 0.06 to 0.5 mm or larger. One package was, however, clearly superior to the other two for routine digital analysis. All three packages require additional development before they are suitable for routine analysis of fine roots. Due to the 252 px mm^{-1} resolution ceiling with currently available scanners, the smallest roots for which this level of discrimination is possible is 0.12 mm diameter. For many agricultural and forest species, up to 95% of their total root length is less than 0.1 mm in diameter. It is concluded that both hardware and software constraints currently inhibit the sensitivity of investigations into fine root diameter shifts in response to environmental conditions.

1. Introduction

Fine roots (up to 95% of root system length) are the primary source of water and nutrients for plant growth and development. Although the classic description of the fine root class states that they are those roots less than 2 mm in diameter (Böhm, 1979), most fine roots are much smaller. In the literature, fine roots of hardwoods, crop species, forages, and weeds have been measured down to 0.06 mm diameter (Lyford, 1975; Wright et al., 1999; Zobel, 2005). The fine root classification lumps 95% of plant root length into one generic classification without a demonstration of their physiological or developmental similarity.

Zobel et al. (2006) demonstrated that in chicory, the fine roots can display three different cultivar dependent responses to phosphorus deprivation: first, a reduction in length of one diameter class cluster of fine roots in favor of a second, thinner diameter class cluster; second, a reduction in fine root mass density without a concomitant change in length; third, the reverse of the first type of response. In the first response type, the larger roots averaged 0.86 mm diameter, and the smaller averaged 0.28 mm diameter (approximately 75% of total root length). The images, from which these data were taken, were photographed at 317 dpi (12.5 px mm^{-1}), and, with the diameter class length plotted against a diameter class log scale, the polygonal histogram displayed dips/humps in the curve, sug-

* Tel.: +1 304 256 2825; fax: +1 304 256 2921.
E-mail address: rich.zobel@ars.usda.gov.
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gestive of distinct diameter class clusters. Two of these humps appeared to correspond with the 0.28 and 0.86 mm diameter class clusters. Similar dips and humps in histograms have been observed with three other species (Zobel, 2005).

The finest roots (0.28 mm diameter) in the Zobel et al. (2006) data averaged 3.5 pixels in diameter. This resolution is, then, slightly below the threshold suggested by Zobel (2003) as that needed to accurately identify distinct roots. Using Zobel's (2003) rule of pixel size needing to be 25% or less than the diameter of the smallest root for accurate identification, a minimum imaging resolution of 0.015 mm (67 px mm^{-1} or 1700 dpi) would have been needed to accurately identify these roots. Subsequent studies of the three chicory cultivars in soil (pots) and field conditions confirm the observations of Zobel et al. (2006), and extend them by demonstrating that the finest roots also change in diameter in response to changes in phosphorus (Zobel, unpublished data).

Ryser and Lambers (1996) and Ryser (1998; cf. p. 452—Fig. 7) present several root histograms that appear to show shifts in *Dactylis glomerata* L. (Orchardgrass, OG) and *Brachypodium pinnatum* (L) Beauv. (Heath False Brome, HFB) root diameter with shifts in nutrient level. These apparent shifts are on the level of 10–30% of the nominal diameter of the roots. Since OG and HFB both have the majority of their fine roots in the 0.1 mm diameter range in field grown plants, a 10% shift in diameter (0.01 mm × 0.25 = 0.0025 mm) requires an optimum resolution of 400 px mm^{-1}. The best resolution for scanners in reasonable price ranges is currently 252 px mm^{-1}. From a visual interpretation of the Ryser (1998) histograms, it might be suggested that OG fine roots become thicker with increased nitrogen, while HFB initiates a new, additional, diameter class of root (approximately 30% larger) with increased nitrogen. Either pattern would require discrimination at the 133 px mm^{-1} resolution level. For species with fine roots in the 0.06 mm diameter range, the required resolution would be on the order of 222 px mm^{-1}, minimum, for a 30% change in diameter. Zobel et al. (2007) demonstrate fine root diameter shifts with changes in nutrient concentration for 12 different species. In their data the diameter shifts averaged 20–25% of the diameter at the lowest concentration. Diameters of the fine roots of the 12 species ranged from 0.07 to 0.27 (Zobel et al., 2007). If shifts in diameter are ubiquitous responses to changes in root environment, as suggested by Zobel et al. (2007), rhizobotanical research will need scanners with significantly higher resolutions, and software capable of analyzing the resultant images.

There are many software packages capable of assessment of root length and average diameter from scan or photograph images. However, very few packages assess the allocation of root length amongst diameter classes down to the pixel level. If root diameter is a continuous variable and roots of different diameter are physiologically identical, this does not matter, but as seen in the Zobel et al. (2006) analysis and as suggested by the data in the Cahn et al. (1989), McCully (1987), Varney et al. (1991), Ryser (1998) and Zobel et al. (2007) research, root diameter is probably not a continuous variable and root physiology may differ with diameter class. The working hypothesis in this laboratory is: root diameter is a discontinuous variable with different meso-diameter classes (clusters of adjoining diameter classes) having distinctly different functional patterns. Roots initiated and growing at different times during the life cycle of a root system will have different diameters due to differences in their growing conditions, and life cycles. If analysis is not carried out at a high enough resolution, such a situation would give rise to an apparent continuous distribution that masks the underlying sets of discontinuous distributions. This leads to the conclusion that for detection and analysis of a discontinuous root diameter distribution, researchers need imaging technology and software capable of dissecting root diameter into diameter classes well into the micron sized pixel range.

Two software packages [WinRhizo v. 2005b (regent.qc.ca) and Delta-T Scan v 2.0 (delta-t.co.uk)] report results as diameter class length, with diameter class set by the user (from multiple pixels per diameter class to actual pixel size/density). Delta T Scan is a legacy software that runs under DOS. Bouma et al. (2000) treated these two commercial packages to a sensitivity analysis of their ability to measure root length and diameter distribution. Their results conclude that the two packages are effectively equivalent and do a good job of assaying diameter distribution. Unfortunately, the highest resolution used in the Bouma et al. (2000) study was 19 px mm^{-1}. According to the analysis by Zobel (2003), this resolution restricts root separation and detection to roots greater than 0.21 mm diameter. A third package, Image Processing Tool Kit (reindeergraphics.com), has several routines that can be combined to give assessments of root length and diameter. One set thins the image to a single pixel thick line then assesses the distance from the line to the edge. Although it does this on a pixel-by-pixel basis, it reports the results as a root segment ("feature") length and mean radius with standard deviations. WinRhizo also uses thinning with threshold edge discrimination, while the actual process used by Delta T Scan is unclear.

The following research is an attempt to test a hypothesis for the most extreme case: existing available hardware and software can detect 10% diameter shifts in roots of 0.06 mm diameter. If this hypothesis is validated, it can logically be assumed that shifts in diameter of larger roots can also be documented. It can be argued that analysis of precise lines of a given thickness is not comparable to the analysis of inherently variable roots. However, any software claiming to be accurate in the assessment of root diameter must be extremely accurate with images with little or no inherent variability. Therefore, in an attempt to determine if current diameter class assessing software were up to the tasks outlined above, we constructed a series of straight lines at many angles and with specific uniform thicknesses. We then analyzed these lines to determine software suitability. This was followed by an assessment of scanned images of wires and roots.

2. Materials and methods

Two different computer systems were used: a Dell[1] Optiplex GX270 with a Pentium 4 at 3.2 GHz with 2 GB RAM, running Windows XP Professional (DELL) and a 2.5 GHz Power Mac G5

[1] Reference to a manufacturer or trademark is solely for the reference of the reader and is not a recommendation by the USDA.