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DigiRoot™: New Software for Root Studies

D. Stefanelli, Y. Fridman and R. L. Perry
(Michigan State University, Department of Horticulture, East Lansing, Michigan, USA)

Summary

The study of root growth and development is a difficult branch of research due to the lack of accurate methodologies. All the approaches used until now to study root dynamics involve a profound disruption of the root environment causing approximations that compromise research goals. Before the technique reported here was developed, there were few commercially available software packages able to identify, measure and report root parameters as extensively. The scope of our work was to design software that would generate accurate and comprehensive data sets of root parameters such as number, length, diameter, area, volume, and branch number, angle, and hierarchy. Our aim was to generate accurate data at a lower resolution than the 12 pixels mm^{-1} (300 dots per inch) currently considered the minimum from several authors. DigiRoot™ is software for measurement of roots in any digital image format. The effects of resolution, orientation of the roots and method of image acquisition (scanned or photographed) on the software's accuracy were tested using objects of known diameters, lengths and angles. Of the previously mentioned test parameters, the only statistically significant effect was that of image resolution on root diameter. With minimal user interaction, DigiRoot™ accurately detects and analyzes roots as narrow as one pixel. The accuracy of the software decreases with decreased resolution, however its accuracy is still adequate at 1 pixel mm^{-1} (26 dpi). DigiRoot™ is new software available to researchers for the study of digital images of roots requiring a high level of accuracy even at low resolution. The software also provides results on branching hierarchy and angle of branch insertion.

Key words. accuracy – angle – branch hierarchy – diameter – digital image – length – resolution

Introduction

Despite the growing recognition that roots play a critical role in determining the overall response of terrestrial vegetation to global change, there is still much to study regarding the mechanisms involved (Bakker 1999; Zobel 2003b). Plant roots can alter their nutrient acquisition capacity by adjusting their physiological longevity and their morphological and/or architectural characteristics to meet changes in sink demand (Chapin 1980; Clarkson and Hanson 1980; Clarkson 1985; Fitter et al. 1991; Bouma et al. 2001; Pregitzer 2002; Zobel 2003b). Pregitzer (2002) states that "a more comprehensive understanding of the anatomy, physiology and dynamics of lateral fine root branches must be developed to comprehend the role of fine roots in biogeochemical cycles". However, root study is a difficult branch of research due to the lack of accurate methodologies, which vary in their degree of disruption of the root environment and in their accuracy, causing biases and errors that can compromise research goals.

Commonly used methods, even if slightly outdated due to their destructivity and other downsides, include "sequential coring" (Singh et al. 1984; Lauenroth et al. 1986; Sala et al. 1988; Nadelhoffer 2000) and "in-growth core" (Vogt and Persson 1991; Nadelhoffer and Raich 1992). Recently, the most commonly used methods are rhizotrons and minirhizotrons, which provide a non-destructive method for viewing and directly studying roots through digital images (Nater et al. 1992; Johnson et al. 2001). Common problems of these methods are that they are expensive, time consuming, and their reliability depends on the accuracy of assessing the physiological status of the roots (Hendrick and Pregitzer 1992a, b; Wang et al. 1995; Comas et al. 2000; Tingey et al. 2000), an essential requirement for root studies (Pregitzer 2002). Also, the definition of fine-root is not standardized among various reports and studies. The diameter at which a root is considered a fine-root decreased over time from ≤3 mm (Melillo et al. 1985) to ≤2 mm (Cheng et al. 1990; Hendrick and Pregitzer 1996; Majdi 1996) to ≤1 mm (McCrady and Comerford 1998; Comas et al. 2000; Eissenstat et al. 2000; Pierret et al. 2005). It then becomes essential for researchers to be able to accurately measure root length and diameter (Vamerali et al. 1999; Zeng et al. 2008).

Up until now, research in this area has primarily focused on understanding root growth and mortality and has completely ignored basic lateral branch ontogeny, making it difficult to understand how the form or the plasticity of the branching root system is related to its function or resource acquisition (Pregitzer 2002). Several authors found that fine-roots without branches generally have higher N concentration, respiration rate, a specific root length and a shorter lifespan than similar sized roots with branches (Pregitzer et al. 1998; Eissenstat et

al. 2000; PREGITZER et al. 2002). To continue developing this area of research, it is essential to develop a method to accurately identify and measure morphological root features at sub-segment and branch levels (NATER et al. 1992; PREGITZER 2002). ZOBEL (2003b) confirms this, stating that, "a plant root system is best described as an integration of multiple genetically and anatomically determined functional root classes." Only by frequent, non-destructive sampling using digital images can we achieve a comprehensive understanding of the anatomy and function of the root system.

The major problem in root studies through digital image analysis is the difficulty in accurate identification and quantification of the specific root features, such as length, diameter and lateral branches. This is exacerbated by the interference of extraneous objects in the background such as lighter soil particles, droplets of water, worms, nematodes and insects (VAMERALI et al. 1999; ZENG et al. 2008). Solving this problem requires that roots be unambiguously identified and measured individually in the image (ZENG et al. 2008).

According to PREGITZER (2002) and ZOBEL (2003b), modern root research is in need of methods to separate, identify and quantify each segment of the branching hierarchy, coupled with the increase of non-destructive sampling methods, to be able to better answer the current questions hindering our understanding of fine roots.

Prior to the work reported here, there were few commercially available software packages able to identify most of the root features needed at once and even less were able to measure each of them separately. Additionally, most of the commonly used software packages are limited by image resolution, giving poor accuracy below 12 pixels (p) mm^{-1} (300 dots per inch; dpi) (VORHEES et al. 1980; KASPAR and EWING 1997; BAUHUS and MESSIER 1999; BOUMA et al. 2000; COSTA et al. 2001; KIMURA and YAMASAKI 2001; 2003; ZOBEL 2003a; HIMMELBAUER et al. 2004). However ZOBEL (2008) infers that it is more a problem of image acquisition than software efficacy.

The scope of our work was to design software that would accurately detect and quantify root features with a substantially higher level of sensitivity. Additionally, our goal was to develop software that could yield image analysis results at a lower level of resolution while maintaining a high level of accuracy.

Software description

DigiRoot™ is software for the automated identification and analysis of roots in digital images. It is based on algorithms developed at the University of North Carolina at Chapel Hill (U.S. Patent Number 6,690,816) to identify and follow elongated objects in digital images. The algorithms were originally studied and developed in the field of medical image analysis for the identification of blood vessels and other such anatomic objects (FURST 1999; AYLWARD and BULLIT 2002; FRIDMAN et al. 2003). The similarity in the appearance and structure of blood vessels and tree roots facilitated the adaptation of these algorithms for use in root analysis.

The foundation for this software is an algorithm that automatically tracks the centreline, or "backbone," of an elongated object (in this case, a root). At each step the algorithm computes the x- and y-coordinates of the object's centreline, the width of the object and the orientation of the object. These four parameters are computed using image analysis techniques that detect the object's edges based on gradients in the image. DigiRoot™ uses this algorithm to investigate roots of arbitrary topology in digital images, without regard for method of image acquisition. The software requires minimal user interaction in root identification. The user must click on three points in each root of interest (an approximate mid-point and two end-points) (Fig. 1) and then the software computes the location, width, and orientation of the root, to sub-pixel accuracy, at all points along the centreline (Fig. 2). The software then uses these values to derive a variety of statistics on the root structure. In particular, the number of roots, root lengths, root diameters, root areas, estimated root volumes, number of branches, insertion angles of branches and branching hierarchy. DigiRoot™ then generates a series of report tables, computing and displaying the statistics described above in a variety of formats. These include:

- Data for each individual root in the image. Roots are numbered in the report as well as in the image to allow for easy identification by the user (Fig. 2).
- Data are classified based on user-defined class dimensions. For example, if the user chooses to classify based on 0.2 mm increments in diameter, data will be shown separately for those roots that are less than 0.2 mm in diameter, those roots that are in the range 0.2–0.4 mm, 0.4–0.6 mm and above.
- Data are computed for each level, or order, of branching, accounting for only the branches in the given level.
- Data are computed individually for each root segment, which is each section between branches.

This selection of output tables provides flexibility in the use of the software regardless of application.

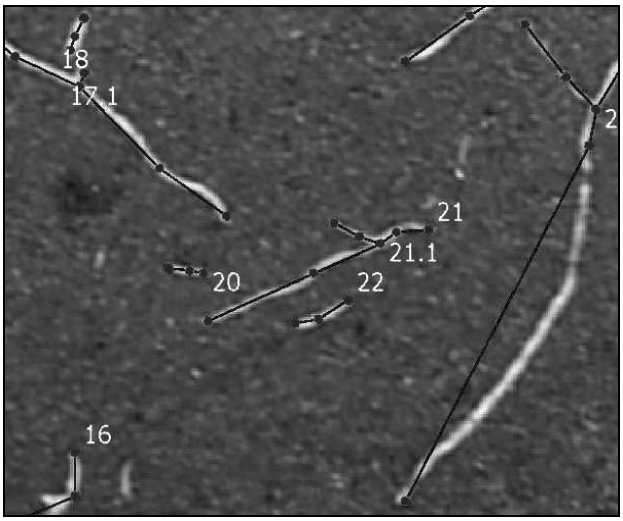


Fig. 1. Snapshot of root selection by the user. Each set of three yellow dots is comprised of the two end-points and the mid-point necessary to identify the roots (i.e., roots 18, 20, and 22) in the image. Roots 17 and 21 show the secondary branch classification and the additional dots necessary for branch selection. Image was acquired using a 2.2 Megapixel Toshiba PDR-M25 digital camera from a rhizotron.

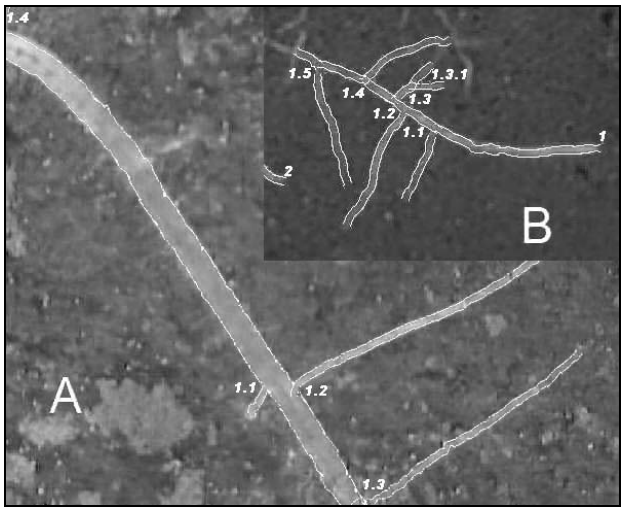


Fig. 2. Snapshot of roots tracked by the DigiRoot™ software. Branch hierarchy is expressed by the incremental secondary numbering. Image A shows one main root with four first order branches (1.1 to 1.4). Image B shows two main roots; root 1 has five first order branches (1.1 to 1.5) and one second order branch (1.3.1). Image A was acquired using a standard Bartz Technology digital camera from a minirhizotron. Image B was acquired using a 2.2 Megapixel Toshiba PDR-M25 digital camera from a rhizotron.

Material and Methods

Software validation

To validate the software, we generated a series of printed images containing a variety of linear segments meant to simulate roots. These linear segments were of varying diameters (0.05, 0.176, 0.353, 0.706, 1.0, and 1.411 mm) and a fixed length (5 cm). The linear segments were printed horizontally, vertically and diagonally on the page to determine if the spatial orientation affected accuracy. Ten lines for each diameter were printed on A4 sheets and used as repetitions with a total of 60 lines per page to simulate different root populations. One sheet was printed for each line orientation. Branching roots were simulated using linear segments branching at different angles (15, 30, 60, 90, and 120 degrees). For angle identification, a constant diameter (0.706 mm) was maintained. The same technique was used to simulate branch hierarchy by adding a second order of linear segments to the previously described images. In this case, both diameter (0.706 mm) and angle (45 °) were constant. Each segment length during the angle and hierarchy measurements was fixed at 5 cm. Ten "branches" for each angle were printed on A4 sheets and used as repetitions with one angle per page. The lengths, diameters and angles of the linear segments were known to high precision and authenticated by the Michigan State University Instructional Media Center.

Methods of image acquisition

The images were scanned with an HP Scanjet 5300C at different resolutions: 1, 2, 3, 4, 12, 24, 35, and 47 p mm^{-1} (26, 50, 72, 150, 300, 600, 900, and 1200 dpi, respectively). These scanned images were analyzed with the DigiRoot™ software and the results compared with the known values of length, diameter and branching angle. Utilizing the Zobel (2003a) table for the correlation between scanned and photographed pixel resolutions, we evaluated the same images taken with a 2.2 Megapixel Toshiba PDR-M25 digital camera. Varying the distance (according to Zobel 2003a) of the camera from the printed images, we were able to obtain photographed images of corresponding resolutions to the scanned images. The same parameters (length, diameter, angle of insertion, and orientation on the page) measured from scanned images were also measured from photographed images.

Statistics

All statistical analyses were performed using SAS (Version 8, SAS Institute, Cary, NC, USA). For the effect of image acquisition method on each parameter measured and each resolution obtained, a variance analysis was performed using PROC MIXED, with the variable being the image acquisition method. In case of acceptance of the hypothesis, all the data were pooled, independently of the acquisition method, and analyzed for the subsequent parameters of length, angle of insertion, orientation on the page, and diameter. For the length parameter, in which the original value was constant, analysis of variance was performed using PROC MIXED, considering as treatments the true values and the measured values at the different resolutions. To determine the effect of orientation, two variance tests were performed: one considering the orientation as treatments, grouping together the resolutions; and a second grouping, which includes the orientations, considering resolution as treatment. In case of acceptance of the hypothesis, percentage errors and standard deviations between the measured results and the true values were calculated. For diameter and angle of insertion, separate analyses of variance were performed using PROC MIXED for each true value, considering the resolution as treatment. In case of acceptance of the hypothesis, all the resolution data were combined

Table 1. Significance (Pr>F values) of resolution, orientation and method of image acquisition (scanned or photographed images) on length, diameter and angle as measured using the DigiRoot™ software.

Root parameter	Resolution	Orientation	Method of image acquisition
Length	0.9246	0.8265	0.8517
Angle	0.7491		0.6248
Diameter	<0.0001	0.3528	0.7592

and a single regression analysis between true and measured data performed. In case of rejection of the hypothesis, different regression analyses between true and measured data were performed for each resolution. Regression analyses between true and measured values for the diameter and angle parameters were obtained using PROC REG for linear regressions.

Results

Image acquisition

There was no significant effect of the image acquisition method (digital photograph or scanned image) on any of the parameters measured (Table 1). Consequently, all the data obtained with the two methods were pooled. Table 1 also shows that the orientation of the linear segments in the image had no statistical effect on length and diameter measured.

Image resolution

There was also no statistical effect of the image resolution (p mm^{-1} or dpi) on length and angle measured (Table 1, Fig. 3). Furthermore, the overall average percentage error between the measured and true lengths was 1.46±0.81 and a very high correlation (adjusted $r^2 = 0.97$ $P<0.001$) was found between the measured and true angles (Fig. 3). However, Fig. 3 also shows that at smaller angles there is a higher estimation error.

One pixel is the minimum diameter accurately measurable, independent of resolution, with the DigiRoot™ software. For diameters between 1 and 2 pixels, some adjustment depending on resolution is deemed necessary, while for diameters from 2 pixels onward, results are very accurate (Table 2, Fig. 4). The overall correlation between true pixel diameter and measured pixel diameter, without regard for resolution, is very high (Fig. 4). As expected and as shown in Table 2 and in Fig. 4, diameters are computed more accurately at higher resolutions than 2 p mm^{-1} (50 dpi).

Discussion

Image acquisition and root length

One of the principal benefits of the DigiRoot™ software is that accuracy is independent of image acquisition method (Table 1). The software does not discriminate once the image is digitized and only considers the resolution.

DigiRoot™ was found to be very accurate in measuring root length. In the literature, most of the effort in research is on the accuracy of the available software (NIH Image, RhizoTron, RIPL, RootEdge and Win or MacRhizo) on length estimation by itself or related to a particular diameter (Vorhees et al. 1980; Kaspar and Ewing 1997; Bauhus and Messier 1999; Bouma et al. 2000; Costa et al. 2001; Kimura and Yamasaki 2001; 2003; Zobel 2003a; Himmelbauer et al. 2004). DigiRoot™ software

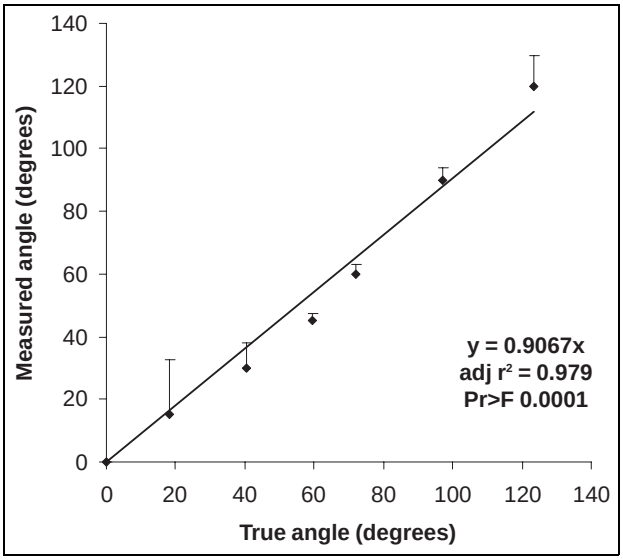


Fig. 3. Correlation between true and measured angles using DigiRoot™. Error bars represent standard deviations. Correlation line and significance was obtained using SAS (Version 8), PROC REG by comparing the averages of the 10 repetitions for each class of angle measured (from 15 to 120 degrees).

Table 2. Regression equations between true and measured diameters at different resolutions expressed as pixels mm^{-1} or dots per inch (dpi).

Resolution (p mm^{-1})	Resolution (dpi)	Regression equation[z]	Adj r^2	Pr>F
1	26	y = −5.0 + 3.58x	0.57	<0.0001
2	50	y = 0.67 + 0.35x	0.88	<0.0001
3	72	y = 0.20 + 0.68x	0.96	<0.0001
4	150	y = 0.02 + 0.89x	0.99	<0.0001
12	300	y = 0.10 + 0.94x	0.99	<0.0001
24	600	y = 0.04 + 0.93x	0.99	<0.0001
35	900	y = 0.04 + 0.86x	0.99	<0.0001
47	1200	y = 0.04 + 0.94x	0.99	<0.0001

[z]Equations and significances obtained using SAS (Version 8), PROC REG. All of the known and measured diameter values were pooled to determine the regression curves at each resolution.

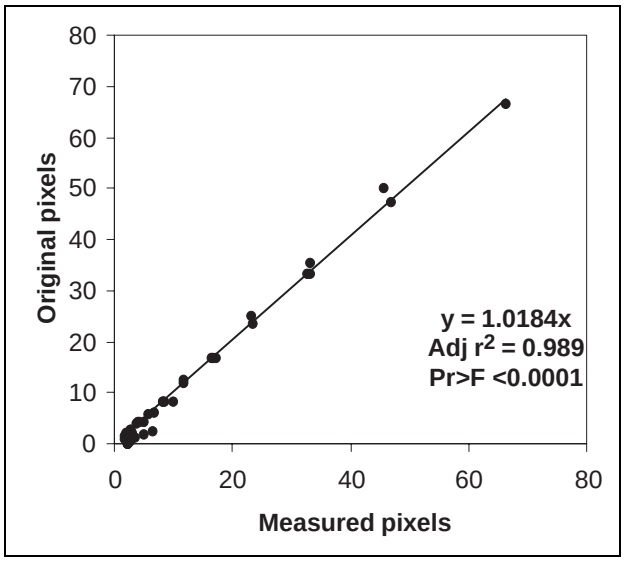


Fig. 4. Correlation between true and measured diameters, in pixels, using DigiRoot™. All the data have been pooled regardless of resolution. Statistics were performed with SAS (Version 8), PROC REG.

does not incur this problem. Errors in length estimation are extremely low and are mainly due to user error. The placement by the user of the two end-points on the root, to let the software know where to start and end measurement (Fig. 1 and Fig. 2), is the main source of error in length estimation. This is shown by the lack of significance of the resolution on length measurements (Table 1). High length accuracy and the ability to divide the roots by their sub-segments based on branch insertions and to divide sub-components based on branch hierarchy solves one of the main problems mentioned by PREGITZER (2002) and ZOBEL (2003b) in their discussions on fine root research.

Angle of branch insertion

The angle of insertion parameter is prone to the same user error mentioned above. Fig. 3 shows that at smaller angles there is a higher estimation error. This is due to the fact that there is a narrower space between the branch and the main root for acute angles and a pixel/size resolution effect (ZOBEL 2008), magnifying the effects of user input placement errors. The high accuracy of angle of branch insertion computation is very useful to researchers (PREGITZER 2002; ZOBEL 2003b), since currently there is few commercially available software that measures this parameter and almost none accurately.

Image resolution

Like other current software, the accuracy of DigiRoot™ is hampered by low image resolution and small root diameters (Table 2 and Fig. 4). There is a notable effect of the resolution on the diameter. Resolution and measured width in pixels are directly proportional, given a fixed root diameter (ZOBEL 2003a; 2008). It has also been shown that there is a range of diameters, depending on the resolution, for which the number of corresponding pixels is less than one. In this case, the measured diameters are always overestimated (KIMURA and YAMASAKI 2001; 2003; ZOBEL 2003a). BAUHUS and MESSIER (1999) determined that the minimum root diameter accurately measurable by the RHIZO software at 12 p mm^{-1} (300 dpi) was 0.5 mm, while ZOBEL (2003a) demonstrated that MacRHIZO can accurately measure root diameters of 0.05 mm at 65 p mm^{-1} (1660 dpi). At the same resolutions, the DigiRoot™ can accurately measure significantly narrower roots; or it can measure the same diameters at lower resolutions (Table 2). Not having done a direct comparison between the different commercially available software packages, we cannot verify their current accuracy and can only draw these conclusions from published results.

Software effectiveness

The high overall correlation between true and measured line diameters (Fig. 4) makes DigiRoot™ a very reliable alternative to the commercially available software for the estimation of this parameter as well. The software does not incur the problem of overestimation and consequently, the need for adjustment of length and diameter when objects were oriented diagonally in the image (ZOBEL 2003a), making DigiRoot™ a useful tool for accurately measuring populations of root diameters in the same image. DigiRoot™ is new software able to generate descriptive data with high precision and comprehensive reports. The software also quantifies parameters that were not measured by existing methods, such as individual root segments, branching hierarchy and angle of branch insertion, that are needed by researchers (PREGITZER 2002; ZOBEL 2003b). This ability gives researchers the capacity to design experiments without concern for source or method of image acquisition. The software does not require calibration, only the image dimensions. DigiRoot™ has been used successfully in research, validating its usefulness in root research and demonstrating its accuracy on actual root images (STEFANELLI 2006). It has been tested on real rhizotron (Fig. 2B) and minirhizotron (Fig. 2A) images with high accuracy of root identification and tracking despite the usual background noise (ZENG et al. 2008). The software was actually developed because of the inability of the current software to accurately identify and track the roots from the rhizotrons images used during the PhD research (Fig. 2B). The software was consequently adapted to track and measure minirhizotron images as well. Additional research is however necessary to further confirm the accuracy of the software working with real root samples from rhizotron and minirhizotron images with different species.

DigiRoot™ is a powerful and accurate tool for addressing the questions raised by leading researchers that need to be overcome to enable a more comprehensive and deeper understanding of root morphology and physiology (See e.g. PREGITZER 2002; ZOBEL 2003b; ZENG et al. 2008). Further research is necessary, especially to reduce the requirement for user inputs and associated errors. This will be achieved through implementing a higher level of automation in the root identification and time frame statistics while maintaining the same level of accuracy.

Appendix

DigiRoot™ is commercially available by Digital Processing Solution, LLC, Divide, CO, USA. It currently runs in Windows operating systems and the code is not available. Copies are available by visiting http://www.digitalprocessingsolutions.com or by contacting the company at info@digitalprocessingsolutions.com.

References

ALYWARD, S.R. and E. BULLITT 2002: Initialization, noise, singularities, and scale in height ridge traversal for tubular object centreline extraction. IEEE Transactions on Medical Imaging **21**, 61–75.

BAKKER, M.R. 1999: Fine-root parameters as indicators of sustainability of forest ecosystems. Forest Ecol. and Mgt. **22**, 7–16.

BAUHUS, J. and C. MESSIER 1999: IMAGE ANALYSIS. Evaluation of fine-root length and diameter measurements obtained using RHIZO image analysis. Agron. J. **91**, 142–147.

BOUMA, T.J., K.L. NIELSEN and B. KPUTSTAAL 2000: Sample preparation and scanning protocol for computerised analysis of root length and diameter. Plant and Soil **218**, 185–196.

BOUMA, T.J., R.D. YANAI, A.D. ELKIN, U. HARTMOND, D.E. FLORES-ALVA and D.M. EISSENSTAT 2001: Estimating age-dependent costs and benefits of roots with contrasting life span: comparing apples and oranges. New Phytologist **150**, 685–695.

CHAPIN, F.S. III 1980: The mineral nutrition of wild plants. Annu. Rev. Ecol. Systematics **11**, 233–260.

CHENG, W., D.C. COLEMAN and J.E. JR. BOX 1990: Root dynamics, production and distribution in agroecosystems in the Georgia Piedmont using minirhizotrons. J. Appl. Ecol. **27**, 592–604.

CLARKSON, D.T. and J.B. HANSON 1980: The mineral nutrition of higher plants. Annu. Rev. Plant Physiol. **31**, 239–298.

CLARKSON, D.T. 1985: Factors affecting mineral nutrient acquisition by plants. Annu. Rev. of Plant Physiol. **36**, 77–115.

COMAS, L.H., D.M. EISSENSTAT and A.M. LAKSO 2000: Assessing root death and root system dynamics in a study of grape canopy pruning. New Phytologist **147**, 171–178.

COSTA, C., L.M. DWYER, C. HAMEL, D.F. MUAMBA, X.L. WANG, L. NANTAIS and D.L. SMITH 2001: Root contrast enhancement for measurement with optical scanner-based image analysis Can. J. Bot. **79**, 23–29.

EISSENSTAT, D.M., C.E. WELLS, R.D. YANAI and J.L. WHITBECK 2000: Building roots in a changing environment: implications for root longevity. New Phytologist **147**, 33–42.

FITTER, A.H., T.R. STICKLAND, M.L. HARVEY and G.W. WILSON 1991: Architectural analysis of plant-root systems. 1. Architectural correlates of exploitation efficiency. New Phytologist **118**, 375–382.

FRIDMAN, Y., S.M. PIZER, S.R. AYLWARD and E. BULLITT 2003: Segmenting 3D branching tubular structures using cores. Medical Image Computing and Computer Assisted Intervention **2879**, 570–577.

FURST, J. 1999: Height Ridges of Oriented Medialness. Ph.D. Thesis, University of North Carolina at Chapel Hill. USA.

HENDRICK, D.L. and K.S. PREGITZER 1992a: Spatial variation in tree root distribution and growth associated with minirhizotrons. Plant and Soil **143**, 283–288.

HENDRICK, D.L. and K.S. PREGITZER 1992b: The demography of fine-root in a northern hardwoods forest. Ecol. **73**, 1094–1104.

HENDRICK, D.L. and K.S. PREGITZER 1996: Application of minirhizotrons to understand root function in forest and other natural ecosystems. Plant and Soil **185**, 293–304.

HIMMELBAUER, M.L., W. LISKANDL and F. KASTANEK 2004: Estimating length, average diameter and surface area of roots using two different Image analyses systems. Plant and Soil **260**, 111–120.

JOHNSON, M.G., D.T. TINGEY, D.L. PHILLIPS and M.J. STORM 2001: Advancing fine-root research with minirhizotrons. Environm. and Exp. Bot. **45**, 266–289.

KASPAR, T.C. and R.P. EWING 1997: ROOTEDGE: Software for measuring root length from desktop scanner images. Agron. J. **89**, 932–940.

KIMURA, K. and S. YAMASAKI 2001: Root length and diameter measurement using NIH Image: application of the line-intercept principle for diameter estimation. Plant and Soil **254**, 305–315.

KIMURA, K. and S. YAMASAKI 2003: Accurate root length and diameter measurement using NIH Image: use of Pythagorean distance for diameter estimation. Plant and Soil **234**, 37–46.

LAUENROTH, W.K., H.W. HUNT, D.M. SWIFT and J.S. SINGH 1986: Reply to Voght et al. Ecol. **67**, 580–582.

MAJDI, H. 1996: Root sampling methods – application and limitation of minirhizotron technique. Plant and Soil **185**, 255–258.

MCCRADY, R.L. and N.B. COMERFORD 1998: Morphological and anatomical relationships of loblolly pine fine-roots. Trees **12**, 431–437.

MELILLO, J.M., K.J. NADELHOFFER and J.D. ABER 1985: Fine-roots, net primary production, and soil nitrogen availability: a new hypothesis. Ecol. **66**, 1377–1390.

NADELHOFFER, J.W. and K.J. RAICH 1992: Fine-root production estimates and belowground carbon allocation in forest ecosystems. Ecol. **73**, 1139–1147.

NADELHOFFER, K.J. 2000: The preferential effects of nitrogen deposition on fine-root production in forest ecosystems. New Phytologist **147**, 131–139.

NATER, E.A., K.D. NATER and J.M. BAKER 1992: Application of artificial neural system algorithms to image analysis of roots in soil, I. Initial results. Geoderma **53**, 237–253.

PIERRET, A., C.J. MORAN and C. DOUSSAN 2005: Conventional detection methodology is limiting our ability to understand the roles and functions of fine roots. New Phytologist **166**, 967–980.

PREGITZER, K.S., M.J. LASKOVSKI, A.J. BUTRON, V.C. LESSARD and D.R. ZAK 1998: Variation in sugar maple root respiration with root diameter and soil depth. Tree Physiol. **18**, 665–670.

PREGITZER, K.S. 2002: Fine root of trees – a new perspective. New Phytologist **154**, 267–273.

PREGITZER, K.S., J.L. DEFOREST, A.J. BURTON, M.F. ALLEN, R.W. RUESS and R.L. HENDRICK 2002: Fine root architecture of nine North American trees. Ecol. Monogr. **72**, 293–309.

SALA, O.E., M.E. BIONDI and W.K. LAUENROTH 1988: Bias in estimate of primary production: an analytical solution. Ecol. Model. **44**, 43–55.

SINGH, J.S., W.K. LAUENROTH, H.W. HUNT and D.M. SWIFT 1984: Bias and random errors in estimators of net root production: a simulation approach. Ecol. **65**, 1760–1764.

STEFANELLI, D. 2006: Evaluation of orchard floor management systems for apple under organic protocol: effect on soil organic matter and nitrogen, nematode community, root architecture and development, and rootstock performance. Ph.D. Dissertation, Michigan State Univ., Dept. of Hort.

TINGEY, D.T., D.L. PHILLIPS and M.G. JOHNSON 2000: Elevated CO_2 and conifer roots: effects on growth, lifespan and turnover. New Phytologist **147**, 87–103.

VAMERALI, T., A. GANIS, S. BONA and G. MOSCA 1999: An approach to minirhizotron root image analysis. Plant and Soil **217**, 183–193.

VOGT, K.A. and H. PERSSON 1991: Measuring growth and development of roots. In: LASSOIE J.P. and T.M. HINCKELY (eds.): Techniques and approaches in forest tree ecophysiology, 477–501.

VOORHEES, W.B., V.A. CARLSON and E.A. HALLAUER 1980: Root length measurement with a computer-controlled digital scanning microdensitometer. Agron. J. **72**, 847–851.

WANG, Z., W.H. BURCH, P. MOU, R.H. JONES and R.J. MITCHELL 1995: Accuracy of visible and ultraviolet light for estimating live root proportions with minirhizotrons. Ecol. **76**, 2330–2334.

ZENG, G., S.T. BIRCHFIELD and C.E. WELLS 2008: Automatic discrimination of fine roots in minirhizotron images. New Phytologist **177**, 549–557.

ZOBEL, R.W. 2003a: Sensitivity analysis of computer-based diameter measurement from digital images. Crop Sci. **43**, 583–591.

ZOBEL, R.W. 2003b: Fine roots – discarding flawed assumptions. New Phytologist **160**, 273–280.

ZOBEL, R.W. 2008: Hardware and software efficacy in assessment of fine root diameter distributions. Computers and Electr. in Agr. **60**, 178–189.

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Addresses of authors: Dario Stefanelli (corresponding author) Department of Primary Industries, Future Farming Systems Research, 621 Burwood Highway, Knoxfield Victoria 3180, Australia, and Ronald L. Perry Michigan State University, Department of Horticulture, A 288 Plant and Soil Science Building, East Lansing, Michigan, USA, and Yonatan Fridman Divide, CO, USA, e-mail: dario.stefanelli@dpi.vic.gov.au.