SOFTWARE

Software for Measuring Root Characters from Digital Images

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ABSTRACT

Root research methods are often tedious, labor intensive, and prone to large variability. Minirhizotron technology has the potential to greatly enhance root research capabilities, but quantifying minirhizotron data is very time consuming. This note presents new software that allows rapid, accurate measurement of root length from digital images—Root Measurement System (RMS). In addition to measuring root lengths and diameters, RMS records number of roots in an image and calculates their total volume, total surface area, and length density. Ten untrained RMS users averaged 654 ± 42 s to measure the length of a 24 mm s-shaped line 10 times. The standard error of the mean for repeated length measurements was <0.1 mm for all but one of the operators. In a subsequent test with 10 different operators having various levels of experience, operators averaged a total of 2324 ± 213 s to measure the lengths and diameters of 10 images of pseudo roots made from wires. There was no significant difference among operators for total length measured, but operators did differ in lengths apportioned among 0.1-mm-diam. classes. For minirhizotron images collected in a field study, an experienced operator could analyze from 17 to 38 images h^{-1} depending on number and length of roots in the images. With its speed, accuracy, and versatility, RMS offers the possibility to analyze sufficient numbers of minirhizotron images to allow detection of treatment effects in field research.

PLANT ROOT SYSTEMS take up water and nutrients from the soil, anchor and support shoots, produce growth regulators, and communicate with shoots to maintain integrated overall plant growth and health. Despite the great importance of roots, research on root systems faces many challenges. Perhaps the two greatest obstacles to root research are difficulty in viewing roots in situ and soil heterogeneity, which leads to large variability in repeated root observations.

While researchers have developed many methods to observe roots, none is without shortcomings. Minirhizotrons have several advantages over other methods for observing roots. Minirhizotron observations are nondestructive, thereby allowing repeated observations of roots to measure root elongation, branching, and turnover as well as root distribution through the soil profile (Cheng et al., 1991; Merrill, 1992; Steele et al., 1997). If tubes are properly installed, soil disturbance is minimal, and results correlate highly with data from soil cores (Bland and Dugas, 1988; Box et al., 1989; Franco and Abrisqueta, 1997). In addition to providing direct observation of roots growing in soils, minirhizotrons also allow observation of other underground plant organs such as rhizomes, peanut (*Arachis hypogaea* L.) pods, and legume nodules and other soil organisms such as insects, worms, and fungi (Lussenhop et al., 1991).

The greatest disadvantage of minirhizotron systems has been the tedious, time-consuming process of translating qualitative information from observations to quantitative data (Hendrick and Pregitzer, 1996). Until recently, most root images have been collected on video, film, or overlays traced with a wax pencil. Root length may be estimated by tracing roots (Beyrouty et al., 1988; Cheng et al., 1991) or by counting root intersections across random transects (Box and Ramseur, 1993; Pietola and Smucker, 1995; Tennant, 1976). Some researchers count roots in the field without storing images for analysis (Franco and Arbisqueta, 1997; Merrill, 1992). One tool that may speed image analysis is automatic computer analysis. Automatic image analysis works well for clean roots (Dowdy et al., 1998; Kimura et al., 1999; Murphy and Smucker, 1995), but background noise makes it much more difficult for roots growing in soils (Bakic et al., 1996).

Recently, there have been great advances in quality and reductions in costs for digital image capture through scanners, digital cameras, or video image capture. Pateña and Ingram (2000) described an inexpensive system for capturing and analyzing digital images collected with a minirhizotron camera. This method used Mouse-O-Meter freeware (Hotware, 29a, rue de Mersch, L-8293 Keispelt, Luxembourg) to convert cursor movement to distance traveled. The authors estimated root length by tracing along roots in an image. Accuracy of this method depends greatly on hand–eye coordination of the operator and length of roots in an image. By this method, long roots are much more difficult to trace accurately than short roots that can be traced while the operator rests his or her hand in a single position.

The objective of this note is to present and assess new software that allows rapid, accurate measurement of root length, diameter, and numbers from digital images.

MATERIALS AND METHODS

Principles and Software Operation

A program called Root Measurement System (RMS; The Univ. of Georgia, Athens) Version 1.5 was written in Visual Basic for MS Windows 95 (Microsoft Corp., Redmond, WA) or higher to measure length and diameter of roots from digital images. For installation, RMS requires 10 megabytes of hard-disk space. Though RMS Version 1.5 accepts only images of

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Abbreviations: RMS, Root Measurement System.

640 by 480 pixels in JPEG format, a revised version under beta testing accepts images of all sizes. Readers interested in using RMS should write to the corresponding author requesting instructions on how to obtain a free compiled copy with documentation through ftp. Though we do not make the source code freely available, we may release specific portions to readers with a specific interest.

The principle of operation is based on the fact that each open computer window has a scale that locates cursor coordinates. In RMS, an open image file fills the 640- by 480-pixel image window so that each cursor position in an image is represented by a coordinate pair. When an operator clicks the mouse button to mark one end of a root segment, RMS records the cursor coordinates. As the operator moves the mouse, a straight colored line appears between the previous click point and the cursor location, allowing the operator to see whether the line remains centered over a segment of root. When the operator clicks a second point along a root, RMS records the coordinates of that point and calculates the distance between points. Once a second point is clicked, the line color changes so that the operator knows which root segments have already been measured. Using calibration factors developed as described below, RMS converts end points of a segment from coordinate units to lengths of objects.

As the user clicks sequential points along a root in an image, RMS estimates root length from the series of segments. By counting the number of times a user ends one root and begins another, RMS also records numbers of roots in an image. To measure root diameter, RMS uses a circular cursor, the diameter of which can be increased or decreased using the + or − keys to match the diameter of each root segment. As the operator changes cursor diameter, RMS uses the new diameter to compute surface area and volume of the segment. Although surface area and volume calculations are based on measurements of diameter to one-pixel resolution, length data are stored in 0.1-mm-diam. classes. (The next version of RMS allows users to set diameter class sizes.) In addition to direct measures of root length and diameter and direct root counting, RMS also calculates root volume and surface area, based on the assumption that root segments are cylinders, and root length density assuming that an image at a planar soil surface represents thin soil layer. The default value of the soil layer for computing root length density is 3 mm (Sanders and Brown, 1978), but users may set this value according to their particular condition and calibration data. For each image, RMS creates an ASCII output file to allow subsequent statistical analysis.

Time Required for Image Analysis

We assessed the speed and accuracy of operating RMS by three methods. First, we asked 10 different people to use RMS to analyze an image of a 24 mm s-shaped line drawn on a piece of paper and captured with a minirhizotron camera (Model BTC-100X, Bartz Technol. Corp., Santa Barbara, CA). To draw a 24-mm line, we first cut a straight piece of wire 24 mm long and then bent the wire into an s-shape and drew a line along the side of the wire with a felt pen. The camera was set to the lowest magnification (about 10×) so that the total area viewed in each image was approximately 19.3 by 13.8 mm. We calibrated RMS before operators analyzed images, and all operators used the same computer for the analysis. Each operator measured the line on the image 10 times, all with the same orientation. Each operator was given the same set of minimal instructions. We assured them that the analysis was neither a race nor a test but requested that they be as accurate as possible. Operators could begin from either end of a line. They were told that they could click as many or as few times along the curve as they wanted but that they should keep the red line stretching from last click to the next between the borders of the image line. Finally, after finishing a trace of each root, operators were to click the right mouse button, select the *end root* option from the menu, and begin the next measurement. For each operator, we collected data on total time for analysis. For each image measurement, we recorded numbers of line segments and total measured length of the line.

In the first test, the line was of uniform diameter along its length, so this test did not allow us to assess the effect of adjusting diameter on speed of operation. A second test was done with 10 different operators having a range of previous experience with RMS. The operators measured both lengths and diameters of pseudo roots for each of 10 images made from bent wires of different lengths and diameters and were instructed on how to increase and decrease circular cursor dimension to match the wire (pseudo root) diameter. Other operator instructions and data collection procedures were similar to those of the first test.

For our third method of assessment, we logged the time required for image analysis from field data sets used in the calibration test described below. A single technician measured length of tall fescue (*Festuca arundinacea* Schreber) roots from images collected at different depths from 32 minirhizotron tubes. In each sample date, a total of 920 images were collected. Numbers of images analyzed each day and time spent in that analysis were recorded.

Calibration

We printed a 20- by 40-mm grid with 1- by 1-mm hatches using a laser printer. We used digital calipers to assure correct dimensions. The grid was wrapped around the outside of a clear acrylic tube of the same diameter as our minirhizotron tubes. We took an image of this grid with the minirhizotron camera before sampling each of 32 minirhizotron tubes installed in field plots of tall fescue.

To calibrate RMS, we first open the image file with a grid of known dimensions. During calibration, RMS uses a cross-hair cursor to aid in aligning the cursor with an intersection of the grid. Then, by first clicking on a grid intersection on the left side and then on the right side of the grid, a line segment is drawn from left to right along a horizontal line of known length, 18 mm in our case. Similarly, a line is drawn from top to bottom, 13 mm long in our case. These grid dimensions compare with a total image size of 19.3 by 13.8 mm. After the user enters the actual lengths of these calibration lines, RMS multiplies the horizontal calibration factor by difference between x-coordinate values of sequential points and vertical calibration factor by differences between y-coordinate end point values and applies the Pythagorean equation to convert distance in screen coordinate points to length of an object in an image.

We conducted analysis of variance for horizontal and vertical calibration factors for four sample dates to test the repeatability of measurements. Some grid images were discarded or lost, so this analysis is based on a total of 104 calibration images, all measured by a single technician.

RESULTS AND DISCUSSION

Time Required for Image Analysis

Total time required to measure an s-shaped curve averaged 654 s across the 10 operators (Table 1), a little

Table 1. Average length, average number of segments, and time for 10 operators to measure the length of a curved line 10 times using Root Measurement System (RMS).

Operator	Length ± SE	Segments ± SE	Time ± SE
	mm	no.	s
1	23.87 ± 0.10a*	29.20 ± 2.12d	620
2	23.79 ± 0.03ab	44.44 ± 1.71b	610
3	23.75 ± 0.03ab	24.00 ± 1.34de	540
4	23.69 ± 0.10ab	39.80 ± 1.52bc	895
5	23.56 ± 0.05ab	52.50 ± 1.20a	585
6	23.47 ± 0.06ab	27.00 ± 1.63de	580
7	23.43 ± 0.06ab	28.70 ± 1.47de	600
8	23.33 ± 0.10ab	37.10 ± 0.90c	620
9	23.10 ± 0.05b	23.70 ± 3.55e	615
10	20.85 ± 0.67c	24.73 ± 1.98de	910
All	23.26 ± 0.07	32.92 ± 0.60	654 ± 42

* Within a column, means followed by the same letter are not significantly different at $P = 0.05$ by LSD.

more than 1 min per image. Operators measured the line without previous practice and with minimal instruction, yet the standard error of the mean for repeated length measurements by an operator was ≤0.1 mm for all but Operator 10. Average length for all operators (23.3 mm) underestimated the nominal length, but we believe this difference arose from making the image rather than measuring it. If we consider the first six measurements as practice and limit our analysis to the final four measurements of each operator, average length increased to 23.7 mm. Thus, it takes very little practice for operators to become proficient in use of RMS.

Operators differed significantly in numbers of segments they used to measure root length (Table 1), and differences among operators remain significant even if we omit the first six *practice* measurements. Surprisingly, there were no significant correlations among time to measure images, numbers of segments, length, or variability of lengths measured (data not shown). Thus, frequency of clicking along the line by operators apparently did not affect either accuracy of length measured or time needed to measure an image.

Length of pseudo roots measured by 10 operators having a wide range of experience did not differ significantly (Table 2). Though operators differed significantly in time required to analyze images and number of segments used to trace roots (Table 2), there was no significant correlation among time for analysis, number of segments used, or total length. Operators with more experience using RMS analyzed images in about half the time of the slowest inexperienced operators. Experience with RMS was probably not the only factor involved; basic familiarity with computers and mouse applications may also explain some of the differences in speed among operators. In addition, when analyzing images of roots growing in soil, it will be important for operators to have training to discern what is and is not a root and whether a brown root is dead or alive. There is no simple rule for relating root color to function or viability. We have observed white branch roots growing from apparently dead and decaying brown nodal roots of tall fescue.

Table 2. Total root length, number of segments, and time for 10 operators using Root Measurement System (RMS) to measure lengths and diameters of pseudo roots in 10 different images. Values are averages per image across the 10 images.

Operator	Total root length	Segments	Time	Relative experience†
	mm	no.	s	
1	124.0	34.5e*	160.7e	+++
2	123.9	61.5a	303.4ab	
3	123.5	51.5bc	294.8b	
4	123.5	48.8bcd	173.6e	++++
5	123.4	52.7b	238.0cd	
6	123.4	37.2e	200.3de	+
7	123.2	45.9cd	165.0e	++
8	123.0	43.9d	350.8a	
9	122.8	24.6f	179.2e	
10	122.7	37.5e	258.4bc	
$P > F$ statistic	0.13	0.0001	0.0001	

* Means followed by the same letter are not significantly different at $P = 0.05$ by LSD.

† Relative previous experience with RMS. A greater number of + signs indicate more experience, while operators with no + signs had no previous experience with RMS.

There was relatively high variability among operators for pseudo root length measured in 0.1-mm-diam. classes (Fig. 1a). As cursor diameter increases, RMS increases diameter in one-pixel increments, a difference that can shift a segment from one diameter class to the next. Thus, diameter is subject to error among operators. Part of this variability may be overcome through operator training and experience. It may also be possible to reduce variability by having a single subject analyze all data for an experiment, but this approach does not necessarily reduce bias, it only assures a consistent bias.

Grouping measured lengths into 0.3-mm-diam. classes decreased variability (Fig. 1b), but it also reduces the information available for interpreting root growth and function. We recommend instead that researchers using RMS be aware of the potential for error in diameter measurements so that they may take adequate care in matching the outer edge of the cursor to the root diameter.

Roots in soil do not normally grow in an s-shape pattern, nor do they have uniform diameters along their length as did the wires used in the second test. Within the 19.3- by 13.8-mm observation area of our minirhizotron images, most roots are nearly straight. There is great variation in numbers of roots in different minirhizotron images depending on soil depth and time after planting. Using images of tall fescue roots under field conditions, we found that one technician could analyze up to 38 images h^{-1} early in the season when roots were few and many images had no roots at all. Later, as root length increased to an average of 34 mm $image^{-1}$, with some images having >200 mm of roots, the same technician could analyze only 24 images h^{-1} on average.

Other factors that may slow measurements include background *noise* in images that may be difficult to distinguish from roots, frequent changes of root diameter, and operator fatigue. Ability to distinguish between roots and other soil features depends mostly on experience. At the same time, one of the strengths of RMS compared with automatic image analysis is that it uses the superior capacity of the human to make such distinctions. Measuring roots of similar size in sequence minimizes the time spent increasing and decreasing the cursor circle to match root diameter. Though it is possible for an operator to measure roots with RMS for a full

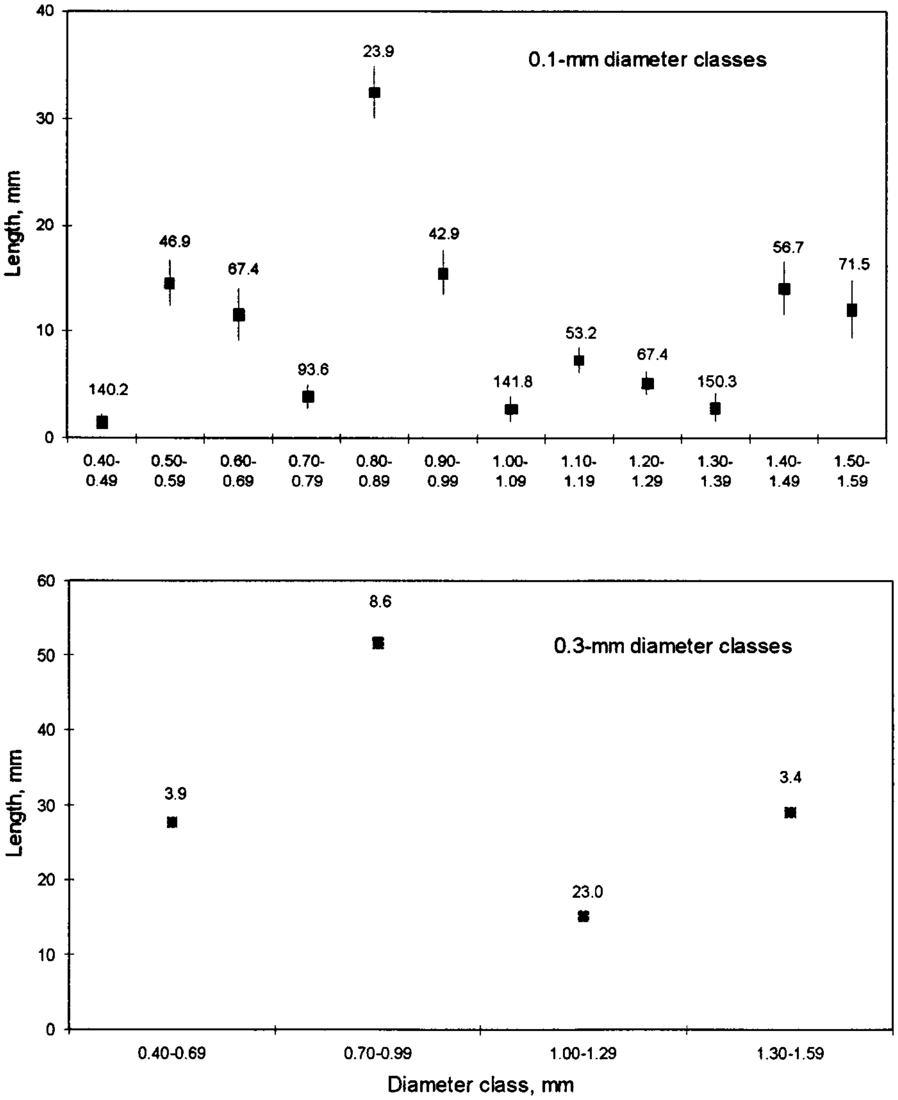

Fig. 1. Length of pseudo roots measured by 10 operators for 10 different images. Each point shows the average across images and operators for length apportioned into (*a*) 0.1-mm-diam. classes or (*b*) 0.3-mm-diam. classes. Vertical bars represent standard error of means; error bars do not show in (*b*) because they are smaller than the data points. Values above each point represent coefficient of variation in percent.

8-h day, we find that shorter work periods of no more than 4 h per day keep the operator fresh and less prone to error.

Accuracy and Repeatability

For 104 calibration grid measurements, we found an average of 71.49 ± 0.06 points mm^{-1} in the horizontal direction and 71.14 ± 0.05 points mm^{-1} in the vertical direction. The difference between horizontal and vertical calibration factors may result from image distortion, which is why it is important to have both vertical and horizontal calibration factors.

Variance in calibration factors within sample dates was very small, with all standard deviation values <0.84% of means. This error could arise from slight differences in distance from the camera lens to the grid when collecting calibration images or from slight differences in where the operator positions the cross-hair cursor during calibration.

To assure data quality, we recommend recalibration as often as is practically possible. At a minimum, we recommend recalibration at the beginning and end of every sample day and each time before and after the camera zoom setting is changed. A good record of calibration factors will help operators evaluate their experimental methods and may identify problems in minirhizotron hardware before they become serious.

Differences between Root Measurement System and Other Methods

There are several other methods that researchers have used to analyze images from minirhizotrons, including root counts, estimating root length by Tennant's (1976) line-intercept method, using a linear probe (tracing wheel) to trace roots on a photograph or video screen, and automatic image analysis. Whether in situ or from images, roots may be counted in less than half the time required to trace roots using RMS. If roots are counted in situ, researchers can save additional time by avoiding the image storage step. The disadvantage of merely counting roots is that the relationships are not constant among numbers of roots and other more physiologically meaningful root measurements such as length, surface area, and volume. Root length in images may be analyzed by the line-intercept method faster than they can be traced by RMS, and using a linear probe takes about the same amount of time as RMS. The disadvantages of the line-intercept and linear probe methods are that neither includes measurement of root diameter and estimation of characteristics that can be computed from length and diameter. Finally, although there are several automatic image analysis systems that work very well for cleaned roots against a uniform background (Kimura et al., 1999; Pietola and Smucker, 1995), none has proven successful in analyzing roots against a soil background.

Because RMS measures root length and diameter and counts roots, its outputs can be compared directly with results from other experiments that have used these other methods. By using the human eye and brain to distinguish roots from other image features, RMS is less subject to error from background noise than are automated systems. Relying on human judgment also allows us to analyze images with very modest computer requirements. Of course, operators do need to learn how to distinguish between roots that are dead and alive and how to handle roots that cross and roots that grow away from and then return to the observation surface.

RooTracker (Duke Univ., Durham, NC) is a software system similar to RMS in many ways. Although a version of RooTracker has recently been released in a beta testing version for Microsoft operating systems, our experience is that it takes much longer to learn to operate and because root length and diameter are measured in separate operations, RooTracker takes as much as twice the time to analyze images as RMS.

Though RMS was designed to analyze root images collected by minirhizotrons, it could be used to measure roots in any digital image, whether from a rhizotron, soil pit, or anywhere else. The only constraint is that either the image must be of known dimensions or a calibration standard must be included in the image. With its speed of operation, accuracy, and ease of learning, RMS makes it possible to analyze sufficient numbers of minirhizotron images to allow detection of treatment effects despite the large variability of most root observations.

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REFERENCES

Bakic, V. 1996. The MR-RIPL 2.0 user's guide [online]. Available at http://rootimag.css.msu.edu/MR-RIPL/MR-RIPL-2.0_guide_HTML/ (verified 13 Mar. 2001).

Beyrouty, C.A., B.R. Wells, R.J. Norman, J.N. Marvel, and J.A. Pillow. 1988. Root growth dynamics of a rice cultivar grown at two locations. Agron. J. 80:1001–1004.

Bland, W.L., and W.A. Dugas. 1988. Root length density from minirhizotron observations. Agron. J. 80:271–275.

Box, J.E., Jr., and E.L. Ramseur. 1993. Minirhizotron wheat root data: Comparisons to soil core root data. Agron. J. 85:1058–1060.

Box, J.E., Jr., A.J.M. Smucker, and J.T. Ritchie. 1989. Minirhizotron installation techniques for investigating root responses to drought and oxygen stresses. Soil Sci. Soc. Am. J. 53:115–118.

Cheng, W., D.C. Coleman, and J.E. Box, Jr. 1991. Measuring root turnover using the minirhizotron technique. Agric. Ecosyst. Environ. 34:261–267.

Dowdy, R.H., A.J.M. Smucker, M.S. Dolan, and J.C. Ferguson. 1998. Automated image analysis for separating plant roots from soil debris elutriated from soil cores. Plant Soil 200:91–94.

Franco, J.A., and J.M. Arbisqueta. 1997. A comparison between minirhizotron and soil coring methods of estimating root distribution in young almond trees under trickle irrigation. J. Hortic. Sci. 72:797–805.

Hendrick, R.L., and K.S. Pregitzer. 1996. Applications of minirhizotrons to understand root function in forests and other natural ecosystems. Plant Soil 185:293–304.

Kimura, K., S. Kikuchi, and S. Yamasaki. 1999. Accurate root length measurement by image analysis. Plant Soil 216:117–127.

Lussenhop, J., R. Fogel, and K. Pregitzer. 1991. A new dawn for soil biology: Video analysis of root–soil–microbial–faunal interactions. Agric. Ecosyst. Environ. 34:235–249.

Merrill, S.D. 1992. Pressurized-wall minirhizotron for field observation of root growth dynamics. Agron. J. 84:755–758.

Murphy, S.L., and A.J.M. Smucker. 1995. Evaluation of video image analysis and line-intercept methods for measuring root systems of alfalfa and ryegrass. Agron. J. 87:865–868.

Pateña, G.F., and K.T. Ingram. 2000. Digital acquisition and measurement of peanut root minirhizotron images. Agron. J. 92:541–544.

Pietola, L.J., and A.J.M. Smucker. 1995. Fine root dynamics of alfalfa after multiple cuttings and during late invasion by weeds. Agron. J. 87:1161–1169.

Sanders, J.L., and D.A. Brown. 1978. A new fiber optic technique for measuring root growth of soybeans under field conditions. Agron. J. 70:1073–1076.

Steele, S.J., S.T. Gower, J.G. Vogel, and J.M. Norman. 1997. Root mass, net primary production, and turnover in aspen, jack pine, and black spruce forests in Saskatchewan and Manitoba, Canada. Tree Physiol. 17:577–587.

Tennant, D. 1976. A test of a modified line intersect method of estimating root length. J. Ecol. 63:995–1103.