Plant root length and diameter determination using an image analysis thinning algorithm

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Abstract

Current techniques for quantitative studies of plant root structures use a statistical grid-intercept method to estimate sample root length, and length:mass ratio to estimate mean root diameter. This study uses a modified image analysis thinning algorithm to measure root length and diameter categories at the same time. The algorithm progressively strips pixels from a binary image of a root, one layer at a time until a single pixel skeleton results. The number of pixels in the skeleton image supplies an estimate of the total root length, while the distance of each skeleton pixel from the background provides the root diameter distribution. Approximately 10 minutes is required to process a single sample including sample preparation and image capture.

Root length estimates obtained using the modified thinning routine were compared with those of the grid-intercept method and were found to differ by no more than 12%. Length distributions for two wires of 0.5 mm and 0.2 mm diameter were estimated with similar accuracy. Percentage of total root length in 4 diameter ranges was estimated for root samples collected from perennial ryegrass plants at different times of the year. Proportion of roots in the finest (0 - 0.3 mm) category varied from 50% in February/March to 35% in July ($p < 0.05$). This seasonal variation confirms previous findings.

***Additional key words:** pixel, quantitative, ryegrass root measurement*

Introduction

Current techniques for quantitative studies of plant root structures use a grid-intercept method (Newman, 1966) to estimate root length and mass:length ratio to estimate mean diameter (Fitter, 1976; Barber, 1971; Barker *et al.*, 1988). Automated root length and area measuring systems based on the grid intercept method (as in Harris and Campbell, 1989) have been available for some years. However, these systems merely imitate the earlier manual methods which only considered the number of intercepts between roots and grid lines (Newman, 1966). In contrast, image analysis considers each pixel systematically, and therefore has the capacity to produce additional information not available when using the grid-intercept method. This paper describes how a modified image analysis thinning algorithm was used to determine root length of samples by diameter categories.

Image Analysis Method.

Computer equipment

The image processing equipment used in this study is based on an IBM PC[1] or compatible computer. A video camera and a video frame grabber are used to capture digital images. The images are stored in the computer's memory and on disk. Image processing software performs both preprocessing and analysis on an image. This equipment is described in detail in the appendix.

Sample measurement

Measurement of samples is performed in three stages:

1. Image capture, in which a digital image of the sample is prepared, the image is captured in a way which enhances the features to be measured.

2. Preprocessing of the image, in which the image is transformed into a form which can be used as data for image analysis procedures.
3. Image analysis, in which the measurements are extracted.

These stages are common to most image processing techniques.

Image capture and preprocessing

Root samples are suspended in water in a shallow glass-bottomed tray against a matt black background with oblique lighting from two directions. This set up produces a digital image (pixel grey values from 0 - 255) with high contrast between root and background. Since the thinning algorithm requires a black and white image the operator chooses a cut off or 'threshold' value. All pixels lighter than the threshold value are converted to white and all darker pixels to black. The operator adjusts the threshold value so that the black and white image looks as much like the original as possible.

A problem encountered at this stage is 'noise' (i.e., small fragments of dust or other material in the background), which would have produced a skeleton of 1-2 pixels and inflated root length estimates. To remove noise, a procedure based on a region filling algorithm described in Foley and van Dam (1982), is carried out prior to thinning. This procedure prompts the operator for the minimum size (in pixels) of an object in the image, and then scans the binary image counting the number of pixels in each object. Objects smaller than the specified area are deleted as noise.

Image analysis

The operating of the thinning transform is conceptually like peeling an onion. One layer of pixels is removed from the edge (border) of the root image, and then the next layer, until only a central axis one pixel wide (which is not removed) remains. The eight neighbours (Fig. 1) of any one pixel are used to determine if it should be removed. Removal of both borders from a two pixel wide object would delete that object entirely. To avoid this, checks are made for pixels which are part of objects which are two pixels wide or are part of 2x2 pixel squares. When a pixel is a part of a two pixel wide object it is deleted only if it is to the right or on the lower part of the line. When a pixel is part of a 2x2 square it is deleted unless it is the pixel in the top left hand corner of the square.

The algorithm used to implement the thinning transform is an adaptation of the HSCP algorithm (after its creators Holt, Stewart, Clint, and Perrot) described in Hall (1989). In this algorithm a boundary layer is removed in three scans through the image. The first scan marks all edge pixels as candidates for deletion. The second checks pixels which have been marked as 'deletable' to see if they are part of two pixel wide lines or 2x2 pixel squares, and 'unmarks' skeletal and other pixels which should not be deleted. The third scan through the image removes those pixels which remain 'deletable'. This process is repeated until no pixels are removed from the image.

The HSCP algorithm was modified so that instead of a pixel being deleted it is given a numerical value indicating the cycle in which it would have been removed. This information is used for the estimation of diameter categories, as described below. After thinning is complete both length and diameter measures are produced in one final pass through the image.

Measurement Methods

The measurements produced by the thinning algorithm are in pixels, and must be converted to millimetres or other units of length. In the case of the equipment used a pixel is a rectangle 4 units wide and 3 units high (Fig. 1). Thus the length of root represented by one skeletal pixel can have one of three values depending on the direction of the skeletal line as it passes through the pixel. Since a pixel has two neighbours in both horizontal and vertical directions and four in the diagonal direction an estimate for the length of a pixel which accounts for the higher probability of traversal by a skeletal line in the diagonal direction (Fig. 1) is:

$$SPL = \frac{1}{4((2 x d5) + d3 + d4)} \qquad 1$$

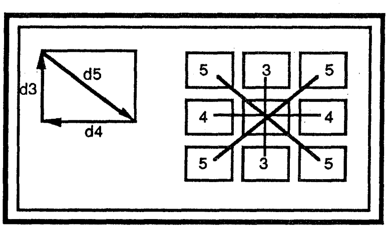

Figure 1: Size of a Pixel and a Pixel's 8 Neighbours. Numbers show relative distances between rectangular pixels of three units height and four units wide.

Where SPL is a 'standardised pixel length' and d3, d4 and d5 represent the linear distance between pixel centres in vertical, horizontal, and diagonal directions respectively. Equation 1 assumes random orientation of roots and would need to be adjusted if placement of roots was non-random.

A calibration command allows the operator to mark any two points on the image and input the horizontal distance between them. This establishes the horizontal distance between pixels (d4 in Fig. 1) which is then used to calculate SPL in millimetres.

Skeletal pixels which remain after the root image has been thinned are totalled by scanning the image and incrementing a counter. The number of skeletal pixels multiplied by the SPL estimates root length. As each pixel is added to the total, its distance from the edge of the root is estimated by averaging the marker values of all non skeletal pixels in its neighbourhood. In this way the total root length is apportioned into diameter categories.

Resolution

A potential difficulty with the method is the resolution of the equipment. For example, to achieve a resolution of 10 pixels per millimetre using a 512 x 512 pixel frame, results in an area scanned of 5 cm x 5 cm. This means that for some applications (e.g., analysing grass roots ranging in diameter from 0.2 - 1.2 mm) determination of diameter categories could be rather coarse. Resolution could be increased, but only by reducing the area scanned, which would limit sample size. Where a requirement for high resolution dictated a small sample size, it would likely be necessary to measure a series of subsamples to ensure consistent results.

Initial Evaluation

Root length estimation by the grid-intercept method and the image analysis technique were compared (Fig. 2). Manual estimates (Tennant 1975) of root length were made on the images of six samples by superimposing a 1 cm square grid over a defined area of each image. The thinning algorithm was then applied to the same area of each image.

The correlation between the two sets of results was 0.97 and the largest absolute difference 12%. These samples were chosen to test a wide range of root lengths.

To test diameter category estimation, an image of two 200 mm coils of wire was used. One coil of wire was 0.22 mm in diameter and the other coil of wire 0.5 mm in diameter. Length estimates for diameter categories 0.0 - 0.3 mm, 0.3 - 0.6 mm and 0.6 - 0.9 mm were 210 mm, 220 mm, and 6 mm respectively. These tests showed that the thinning algorithm was operating correctly, although during testing with the coils it was noticed that diameter categorisation was sensitive to variation in the threshold value applied to the image. However total estimated length varied only slightly over the same range of threshold values (Fig. 3).

Application

Samples of perennial ryegrass root collected at different times of the year were analysed. Field studies (Matthew *et al.*, 1990) have shown that mean diameter of ryegrass roots (calculated by mass:length ratio) shows seasonal variation, with diameter values being greatest in August, and lowest in November - December.

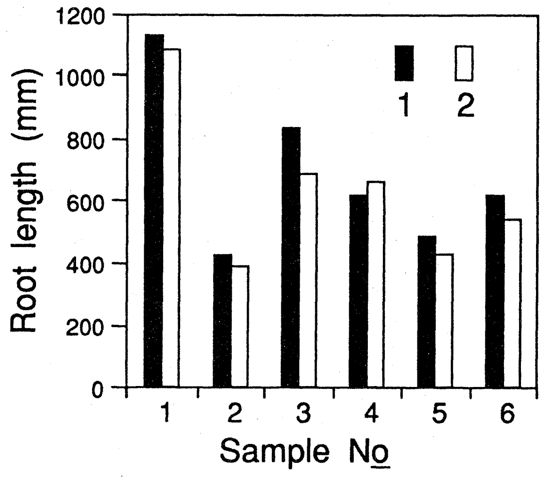

Figure 2: **Comparison with Manual Technique. Six root samples were measured using both techniques. The bars depicted with pattern 1 are length estimates (mm) using the manual technique and the bars depicted as pattern 2 are length estimates using the thinning technique.**

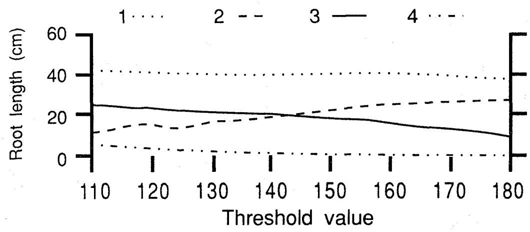

Figure 3: Effect on estimated root lengths with variation in threshold value. ((1) Total root length, (2-4) 0 - 0.3, 0.3 - 0.6, and 0.6 - 0.9 mm diameter categories respectively. SPL approx. 0.1 mm).

Plugs of ryegrass tillers (79 mm diameter, 100mm depth) were transplanted from grazed paddocks to outdoor sand-boxes at 2 monthly intervals from January until November 1989. On each occasion 6 plugs were transplanted and were grown on in sand boxes for 6 weeks, by which time new roots had grown from the transplanted plugs into the sand. Plugs were then harvested by hosing away sand to expose new roots. For 3 plugs new roots were clipped with scissors at the point where they emerged from the plug, and stored for image analysis. The remaining three plugs were used for other measurements.

The percentage of total length of new roots in each of four diameter categories (<0.3mm, 0.3 - 0.6mm, 0.6 - 0.9mm, 0.9 - 1.5mm) over 5 consecutive harvests did show seasonal variation with a greater proportion of roots in the finest category in summer (Fig. 4). These seasonal changes in percentage of total root length in coarse and fine diameter categories were highly significant statistically, and agree well with the previous findings of Matthew *et al.* (1990). The functional significance of these seasonal differences in root diameter is not understood at this time although there is evidence (C. Matthew, unpublished data) that the effect is not attributable solely to changes in the degree of branching. Seasonal change in the diameter of new nodal roots also appears to be involved.

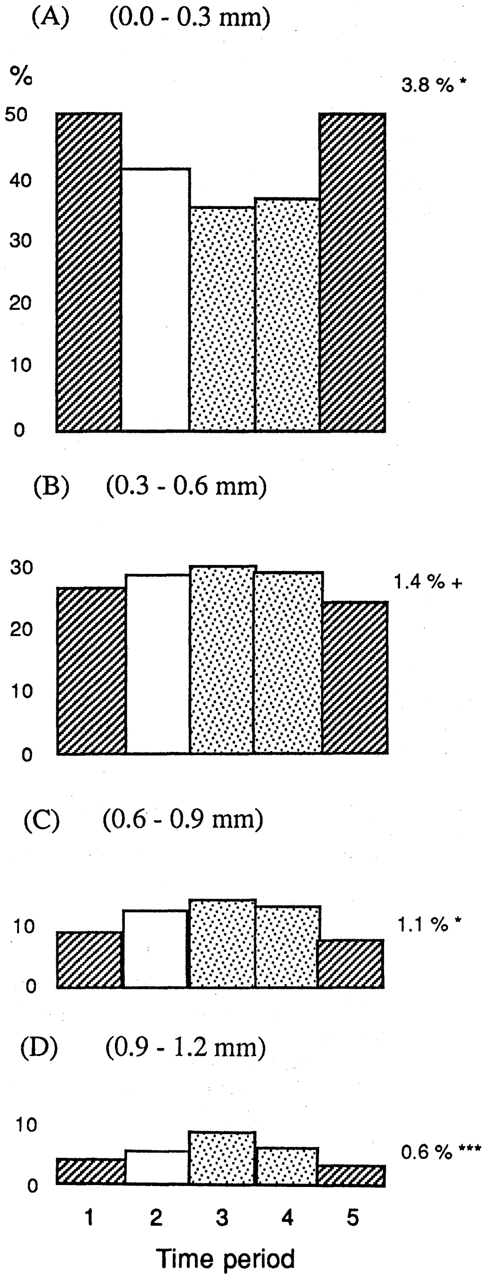

Figure 4: Percentage of total root in four diameter categories (A-D) for five time periods in 1989 (1, Feb/Mar; 2, April/May; 3, July; 4, Aug/Sept; 5, Oct/Nov). SEM are shown at the right.

Further Development

While the software in its present state produces useful information, further development would be desirable. It is suggested that sample processing time could be reduced by an improved thinning algorithm (Arcelli and Sanniti di Baja, 1986) and the versatility of the system could be improved with extensions to the user interface. For example, techniques for the automatic selection of threshold values (e.g. Rosenfeld and De La Torre, 1983) have been developed. Applying an appropriate automatic thresholding technique during preprocessing would reduce inherent operator stress, and improve consistency from operator to operator.

Two technical problems still remain and produce minor errors. First, in some samples there is a small over estimation of root length due to uneven edges of roots producing spurious branches of 1 - 5 pixels length during thinning. Second, where two roots cross, they share a common skeletal line and length is slightly underestimated.

Recent studies (e.g. Fitter, 1990) have shown that root branching pattern has implications for efficiency of root function. A group in the United States (Smucker *et al.*, 1987) has used a thinning algorithm approach to identify branch points on the skeletal axis in addition to root length and diameter measurement. The provision of branching pattern information could be incorporated in the present software or software based on the Arcelli and Sanniti di Baja (1986) algorithm.

Another possible extension is the determination of live:dead ratios of root samples. This could be achieved by comparing images of stained roots, photographed using different filters, or by using colour image processing equipment.

Summary

An automatic method based on image processing techniques has been developed for measuring root length and diameter. Length of root in a sample is estimated by the length of the skeleton of the sample image. The sample is apportioned into diameter categories by taking the distance of each pixel in the skeleton from the edge of each root in the original image. Measurement of seasonal variation in percentage of total root length in fine and coarse diameter categories for perennial ryegrass agrees with previous measurements. With further development the method could be extended to include measurement of branching patterns. At present the method provides a useful tool for the measurement of plant root samples.

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APPENDIX

Computer equipment

The measurement algorithm described in this paper was developed to work with the Imaging Technology PCVISION*plus*[2] Frame Grabber and ITEX[2] software library. The PCVISION*plus* system is an IBM PC or IBM PC compatible card that interfaces with the standard XT or AT bus. This card includes two 512 x 512 x 8 bit pixel frame buffers and facilities for frame grabbing and image display. Companion to the PCVISION hardware is the ITEX PC library, an object code software library that provides a comprehensive suite of functions for controlling all aspects of the frame grabber operations including: acquiring images, reading and writing image data to disk, and reading and writing image data to and from the host computer memory. Central to the image processing system along with the PCVISION frame grabber and ITEX software is a general purpose PC computer. This PC performs vital operations like the numeric computation of the image data as well as storing and retrieving image data to and from disk. All software described was written in Microsoft C[3].

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