PLANT ROOT LENGTH DENSITY MEASUREMENT USING IMAGE PROCESSING

by

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ABSTRACT

A thinning algorithm-based image analysis technique was developed to measure corn root lengths. The root length measurement method was evaluated by comparing thread lengths measured by the image analysis system with actual thread lengths. The length measurement method accurately estimated actual thread lengths (less than 2 % calculated error). Also, a rapid root length density measurement procedure, which utilizes the above root length measurement method, was developed to estimate corn root length density without washing the roots. Root length densities estimated from the cut soil surface of core samples taken from the field were paired with the root length densities determined from washed roots from the same soil core sample. A linear relationship between these two values was expected and was found. Eliminating the root washing procedure reduces the time required for measuring corn root length density substantially.

Key Word : Corn-Roots, Image-Analysis, Root-Length

INTRODUCTION

Plant root growth is sensitive to environmental stresses and to changes in soil properties associated with soil management practices. Plant roots integrate climatic and soil conditions with the genetic potential of the plant. All plants grow and reproduce in response to an interaction of dynamic and ever-changing components in their environment. Maximum growth rate and yield are achieved when these components are in adequate supply (Rendig and Taylor, 1989). Changes in plant root parameters such as length, surface area, or number provide important information for managing soil effectively, studying nutrient uptake and water extraction. However, a small amount of research effort has been devoted to solving the root quantification problem because studying roots requires a great deal of time and labor. Thus, effective root research requires a method for reducing these time and labor requirements.

Most of the existing root observation research has focused on measuring root length because root length is one of the best parameters for calculation of water uptake by plant roots. Also the length of the roots seems to be a good parameter for studying the process of nutrient uptake by plant roots. Another advantage of using root length in root studies is the possibility for rapid determination. These reasons make root length the preferred measure in root studies.

Root length can be measured manually by using a ruler or ruled graph paper. However, these manual measurements are tedious and time consuming. The time required to measure root length is reduced by using the line intersection method.

Line Intersection Methods

This method calculates root length rapidly by counting the intersections between roots and a regular pattern of lines. According to Newman's theory (1966), root length can be estimated by the equation

$$\mathbf{R = \pi AN/2H} \qquad (1)$$

where R is the total length of roots in an observed area A and N is the number of intersections between the roots and random straight lines of total length H. Newman's method has been modified and improved by changing the area over which the roots are spread and the size of the grid system.

Root length measurement methods have evolved significantly by using a certain instrument. A video image analyzer system, called the Delta-T area meter (Decagon Devices, Pullman, Wash.), was used to measure root length in studies of plant roots (Shuman et al., 1993). The device counts the number of intersections between the video camera scan line and the edge of objects in the field of view. The roots are detected by the device on the basis of sharp contrast between the background and the roots. The image of roots, the total scan area measured by the meter, and the meter readout of root length are displayed on the video monitor.

However, the root length measured by the video image analyzer system that utilizes line intersection is affected by scan direction. Furthermore, a small number of the counted number (less than 50) of intersections can decrease the accuracy of the results (Böhm, 1979).

Literature Reviews

Reicosky et al. (1970) compared three methods of estimating root length: the direct method, the inch counter method, and the line intersection method. The line intersection method had a tendency to overestimate the root length.

Cunningham et al. (1989) compared root length estimated by the manually operated modified line intersect method with root length estimated using a video image analyzer (Delta-T area meter). In an experiment in which the lengths of a 0.9 mm diameter wire network were measured, the video image analyzer method was found to be linearly correlated (R^2 = 0.92) with the modified line intersect method.

Harris and Campbell (1989) evaluated the use of an inexpensive commercial image analysis system for measuring the length of roots in samples washed from soil. The analyzer, which consisted of a high-resolution television (TV) camera and a comparator, measured the intersections of the TV scan lines and roots in the view area. Roots were scanned in two directions to minimize errors due to nonrandom orientation. The system was tested using known lengths of thread or string, either formed into circles to simulate random orientation with respect to scan lines, or placed with random orientation on the tray to simulate actual roots. The errors were less than 5% after overlap and resolution correction.

Although a fair amount of research has been done to improve the root length measurement method, more accurate, faster, and less expensive approaches are needed. Using the image processing technique can provide faster and accurate measurement of plant root length. Because the image of roots is stored and processed on a computer, it is possible to automatically obtain the characteristic variables of the root structure.

Objectives

The overall goal of this study was to develop a plant root length measurement system using image analysis. To achieve this goal, the three specific objectives formulated were:

1. To develop an inexpensive image analysis system for measuring plant root length, and
2. To evaluate the use of an image analysis system for measuring plant root length, and
3. To develop a rapid procedure based on image analysis for measuring plant root length density *in situ*.

To accomplish these objectives, an image analysis system which included a thinning algorithm-based length measurement method was developed. Laboratory experiments were conducted to evaluate this system by comparing thread lengths measured by the image analysis system with actual thread lengths. Also a rapid root length density measurement procedure was developed which included image analysis based a length measurement method, a root staining technique, and core sampling.

MATERIALS AND METHODS

Root Length Measurement Method

To overcome the limitation of the root length measurement system that utilizes the line intersect principle, a thinning algorithm-based image analysis method was used to measure root length. In the thinning algorithm-based image analysis method, the overall root image is reduced to its basic skeleton; then the pixels of its eroded skeleton are counted. This method eliminates the problem related to scanning directions which is the main drawback of the root length measurement system that utilizes the line intersection principle.

Skeletonization

Reducing root image to its basic skeleton is called skeletonization. Skeletonization is a binary image transformation that may be implemented using morphologic operation, and results in a one-pixel-thick line with the same topology as the original object. A skeleton representation of an object can be used to describe its structure. Skeletonization helps in the classification of unknown objects within an image, because the amount of complexity of the object is less than when all the pixels within an object are used. Root length can be measured simply by counting the number of pixels in the skeleton only.

Calibration

Image analysis system calibration is necessary to express root length in terms of spatial unit (cm) rather than by number of pixels. A standard ring was used to calculate the ratio in pixels per cm. Because the focal length was slightly changed for every video-recording equipment setup, the resolution in pixels per cm was calculated for every different camera position. The root length was estimated by:

$$\mathbf{RL = r \times N} \qquad (2)$$

where RL = root length estimate, r = ratio in cm per pixel, and N = number of pixels in the thinned root structure.

Estimating Plant Root Length Density *In Situ*

Another time consuming part for measuring plant root dynamics is the root washing procedure. To eliminate this root washing procedure, a root length density measurement procedure which utilizes thinning algorithm-based image analysis method was developed.

Overall Description of the Root Length Density Measurement Procedure

This procedure used a thinning algorithm-based image analysis method to measure root length at the cut soil surface, which is called rooting intensity. A core sampler was used to extract root-soil samples from a corn field. The core sample was cut in half along its length using a knife and the roots were exposed by careful handling. The root-soil sample was placed in a box to protect it from sunlight. The exposed roots faced up toward a video camcorder. Long wave ultraviolet light illuminated the root-soil sample to brighten the live roots selectively. The contrast-enhanced image was acquired by the camcorder. The acquired root image was digitized by a frame grabber and changed to a gray scale digital image. The roots were differentiated from the background by thresholding (which sets every pixel brightness in an image to either black or white depending upon whether the original pixel brightness is below or above a threshold value) the gray scale image, and then converting to a binary image. The roots, now represented by white pixels, were thinned and reduced to basic structure. Root length was calculated by counting the pixel numbers of the root's basic structure and multiplying the pixel numbers by the cm/pixel ratio. The cm/pixel ratio was calculated from the relationship between known circumference of a standard ring and the number of pixels of that circumference in the image. This root length was divided by the area of the profile of the core sample and represented as rooting intensity. The rooting intensity was paired with root length density, measured from a washed root sample also obtained from a soil core from the field.

The root length density measurement followed the same image analysis procedure as the rooting intensity measurement, except that the root image was acquired from washed roots. The root length density was calculated by dividing the root length as measured from the above steps by the volume of core sample.

Root Staining

In order to improve the contrast with the background soil, the roots were stained by autofluorescence. The autofluorescence of corn roots is caused by the presence of a special compound called "coumarin." The coumarin has the characteristic fluorescence upon ultraviolet irradiation (Murray et al., 1982).

To cause corn root autofluorescence, long wave (360 nm) UV light (100W long wave ultraviolet floodlight lamp, Spectroline MB-100, Spectronics Corporation, New York, USA) was used. Also, a UV filter was attached to the television camera lens to increase enhancement.

Image Analysis System Description

The image analysis system consisted of an IBM 486DX/33MHz personal computer equipped with a video frame grabber-digitizer board (Data Translation DT3851), built-in math co-processor, SVGA-compatible video graphics array, 8MB RAM, and a 340MB hard disk. An additional personal computer equipped with a Pentium 100MHz processor, SVGA-compatible video graphics array, 16MB RAM, and a 850MB hard disk was used to perform an intensive image analysis operation in the laboratory.

Root images were acquired by using two different image acquisition systems depending on where the images were taken. A color television camera (CCD TR93, Sony) was used for recording root images in the field. Figure 1 shows a schematic diagram of the root image recording system for the field experiment.

A box was used to block the sunlight and provide dark lighting conditions when recording the root images in the field. An ultraviolet (UV) lamp was used to increase the contrast between roots and background soil. The root images taken by television camera were stored on a videotape in analog form. When all root images were recorded, the analog form of each root image was digitized with the frame grabber board and stored on a computer hard drive in the laboratory. The frame grabber board had a 640×480 spatial resolution with 256 (8 bits) gray-scale levels. The video signal from the video camera was directed to the digitizer producing an 8-bit, 640-horizontal by 480-vertical digital image with 307,200 pixels. A pixel value of 0 was designated as black and a 255 value as white.

A monochrome CCD camera connected to the frame grabber was used for recording washed root images in the laboratory (Figure 2). The images from the monochrome CCD camera were digitized with a video frame grabber board. Then the digitized images were stored on a computer hard drive for later analysis.

Software Description

All digital image processing except thinning and counting of captured root images was accomplished in the Windows environment using Image-Pro Plus developed by Media Cybernetics (Silver Spring, MD, USA). Downloaded root images from the camcorder and captured washed-root images from the CCD camera were enhanced by adjusting brightness, contrast, and gamma value. A Low Pass filter was applied to eliminate spot noise of root images recorded from the field experiment. Enhanced images were converted to black and white images by thresholding to separate roots from the soil background.

After thresholding, the binary root images were skeletonized. The root lengths were estimated by counting the number of white pixels and multiplying by a conversion factor, which was the pixel-to-cm length ratio. The root images were skeletonized and counted by a program called Image Analyzer that was developed with MATLAB (The MathWorks, Inc., Natick, MA, USA) software. The Image Analyzer has a graphical user interface (GUI) made up of graphical objects, such as menus, buttons, and lists. This GUI was implemented to provide easy use and to reduce image handling time.

Analysis

The length measurement system using image processing was checked with a known lengths of white sewing thread. The thread samples were cut into several pieces and dispersed randomly over black paper. Thread images were taken for each sample. These thread images were analyzed and thread lengths were measured. The measured thread length were paired with actual thread to find the linear relationship between them.

To evaluate the root length density measurement procedure, the rooting intensity and root length density values were paired and the relationship between these two values was compared. The root length density measurement procedure was assessed by examining the correlation coefficient of the linear regression equation acquired from the respective pairs of rooting intensity and root length density.

Root Sample Preparation

Corn plant (*Zea mays*) roots were used to obtain samples for estimating root length density. Corn was planted in early May and well irrigated. Soil coreing was done in late July after the root systems were fully developed. To increase the range of root length density values, samples were taken from several different locations. Three corn plants were selected and core samples were obtained from different depths in the soil and distances from the plant. For each corn plant, six core samples were taken. Root samples were taken at 10 cm, 20 cm, and 30 cm away from the corn stalk. At each distance, samples were taken for the two depth ranges 0~13 cm and 13~26 cm.

RESULTS AND DISCUSSION

Evaluation of the Root Length Measurement Method

The length measuring system was evaluated by comparing thread lengths measured by the image analysis system with actual thread lengths (Table 1). The image analysis method estimated approximately the same lengths for the given samples. The high correlation coefficient (0.999) and the close relationship to the 1:1 line (the slope being 0.995) indicate the closeness of the relation between the actual lengths and the estimated lengths derived from the image analysis. The image analysis method appears to be reliable. The calculated errors were less than 2%.

Relationship Between Rooting Intensity and Root Length Density

Analysis of the data set acquired from the field involved calculating correlation coefficients pairing the root length density from washed root samples with the corresponding rooting intensity from the planar soil face. There was a linear relationship between root length density and rooting intensity (Table 2). Table 2 shows the correlation coefficients of the regression equation, calculated from the relationship between rooting intensity on the soil surface and measured root length density from the washed roots. Rooting intensity is highly correlated with root length density ($R^2 = 0.959$).

Estimation of Root Length Density

After the linear relationship between rooting intensity and root length density was found, a direct estimation of root length density from root length at the cut soil face was made. Root length density was calculated by dividing the root length at the cut soil face by the volume of the removed soil layer. Roots were exposed by removing about 2 mm of the soil layer during the field experiment.

Figure 3 shows the relationship between estimated root length density (cm/cm^3) and measured root length density (cm/cm^3) from washed roots. The estimated root length densities calculated from root lengths on the cut soil surface are well correlated with measured root length density from the washed roots (R^2 = 0.959).

The agreement between estimated root length density and measured root length density for corn roots would suggest that rooting intensity of the soil core can be converted to estimates of root length density in the soil. This conversion would reduce the time required for measuring root length, and it may eliminate the need for relatively severe destructive sampling methods.

The time required for estimating root length density from the unwashed root-soil sample by image analysis was significantly less than that required for processing soil cores. The technique proposed in this research required approximately 20 minutes per sample. In contrast, the time required for the conventional method, in which the root lengths are measured from washed samples, to process the soil was about 50 minutes (Böhm et al., 1977). This rate of measurement is nearly 2.5 times greater than previous methods in the literature. Image analysis thus represents a time savings which allows the possibility to collect more root information during a season.

Autofluorescence caused by ultraviolet light effectively differentiated viable corn roots from organic matter and the soil matrix. Corn roots showed blue fluorescence excitation under long wave (360 nm) ultraviolet light. However, the intensity of root autofluorescence was so weak that it was only observed in a dark environment.

This procedure can be used to reliably estimate the root length of root systems in soil with minimal damage to the plant. The method is a useful tool to improve our knowledge of root system development.

CONCLUSIONS

A root length measurement method using a thinning algorithm-based image analysis system was developed and evaluated. Also an image analysis-based root length density measurement procedure was proposed and evaluated. The procedure estimated corn root length density at the planar faces cut from a core sample taken from the field. The estimated root length densities were regressed on root length densities, measured from washed roots in the same soil core sample, and a linear relationship was obtained. Based on above results, the following conclusions are drawn:

1. The thinning algorithm-based length measurement method was accurate enough to use for research in which the accurate length of washed roots is needed.
2. The image analysis root length density measurement procedure reduces the time required for determining the length of corn root systems by eliminating the root washing procedure.

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Table 1. Relationship between measured thread lengths and actual thread lengths.

Regression Coefficient(β_1)	Intercept(β_0)	R Square
0.995	0.54	0.999

Table 2. Relationship between rooting intensities and measured root length densities of bulk soil contained in soil cores.

Regression Coefficient(β_1)	Intercept(β_0)	R Square
5.4271	0.1376	0.9589

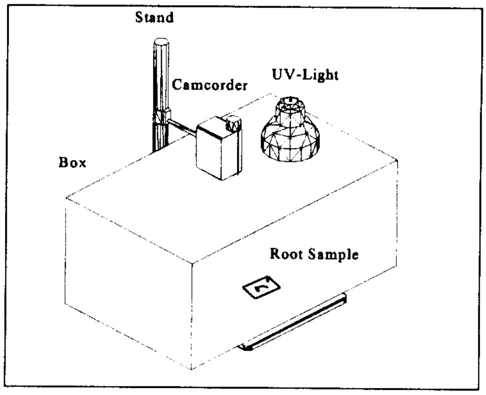

Figure 1. Schematic diagram of the root image recording system.

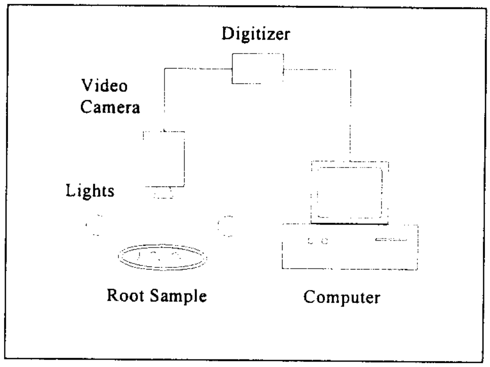

Figure 2. Schematic diagram of the root length measurement system.

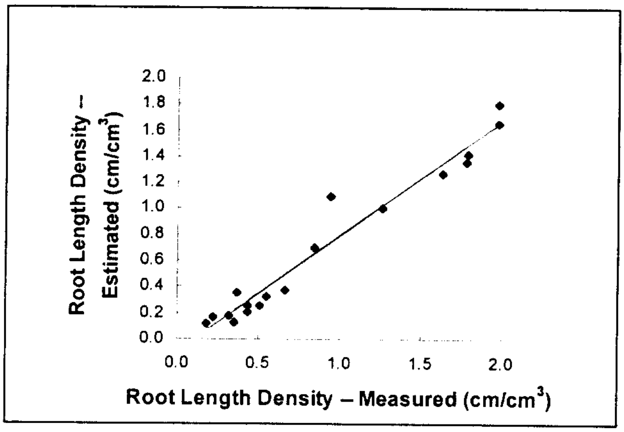

Figure 3. Relationship between estimated root length density (cm/cm^3) and measured root length density (cm/cm^3).