DEVELOPMENT OF ROOT OBSERVATION METHOD BY IMAGE ANALYSIS SYSTEM

by

Giyoung Kim

Dissertation submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Biological Systems Engineering

APPROVED:

David H. Vaughan
D.H. Vaughan, Chairman

Sidney H. Deck
S.H. Deck

D. Earl Kline
D.E. Kline

G.D. Kulasiri

S.J. Thomson

J.V. Perumpral
Department Head

December, 1995
Blacksburg, Virginia

Key Words: Root Observation Method, Image Processing

DEVELOPMENT OF ROOT OBSERVATION METHOD BY IMAGE ANALYSIS SYSTEM

by

Giyoung Kim

D.H. Vaughan, Chairman

Biological Systems Engineering

(ABSTRACT)

Knowledge of plant roots is important for determining plant-soil relationships, managing soil effectively, studying nutrient and water extraction, and creating a soil quality index. Plant root research is limited by the large amount of time and labor required to wash the roots from the soil and measure the viable roots. A root measurement method based on image analysis was proposed to reduce the time and labor requirement. A thinning algorithm-based image analysis method was used to measure corn root length at the planar faces cut from a core sample. The roots were exposed by careful handling and contrasted from the soil by causing autofluorescence using long-wave ultraviolet light. The contrast-enhanced images were stored on the camcorder video tape and digitized by frame grabber. A binary root image was acquired from the digitized gray scale image by a thresholding operation. The binary root image was thinned until the roots were reduced to their basic structure. Root length was calculated from the number of pixels of the root's basic structure. This root length was divided by the removed soil volume of the profile of the core sample to estimate the root length density (RLD, cm root cm^{-3} soil). This estimated RLD was regressed on RLD, measured from washed roots in the same soil core sample, and a linear relationship ($R^2 = 0.96$) was obtained. This study indicated that the image analysis root measurement method can determine the length of corn root systems up to 2.5 times faster than by using the conventional method which incorporates a root washing procedure.

Dedication

To my parents who have made sacrifices, provided moral support, and encouraged my pursuit of an advanced education,

to my parents-in-law whose love and supports are always there,

to my Gumdo Master, Mr. Yun whose continued support and insistence on my being focused on my goal have been helpful and invaluable, and

to my wife who has always been available with her unique sense of humor, understanding, and constant assistance that helped lighten my burden.

Acknowledgments

I would like to express my appreciation to the following members of my doctoral committee: Dr. Sidney Deck, Dr. Earl Kline, Dr. Don Kulasiri, Dr. John Perumpral, and Dr. Steven Thomson. They allowed me the freedom to pursue the ideas that interested me. I am grateful to each for their advice and support throughout this study.

I especially want to thank Leon Alley for his assistance.

Special thanks go to my ever-helpful advisor, Dr. David Vaughan. Throughout the entire course of my study, he has given invaluable support, guidance, and encouragement. Clearly, I would never have finished this dissertation without him.

Table of Contents

List of Figures

List of Tables

I. Introduction

1.1 Plant Roots and Sustainability Index

Soil is one of the most important resources for agriculture. The main future threat to agriculture is soil degradation or a decline in soil quality. In many countries, soils are being degraded by wind and water erosion, desertification, compaction, acidification, sodification, and salinization caused by misuse and improper farming methods. When soil is degraded, the soil's capacity to yield healthy crops is reduced, the soil's resistance to erosion decreases, and the impact of environmental stresses on plants increases. Therefore, preservation of soil quality is essential to support a sustainable agricultural production system.

One solution for preserving the soil is a sustainable agriculture system, a goal that enables the preservation of resources through the increasing utilization of renewable resources. The goal of agricultural sustainability requires the use of efficient farming methods to maintain soil quality and minimize losses of soil. A sustainable agriculture system could obtain optimal production through the application of efficient techniques such as crop rotation, reduced chemical input, cover crops, crop residues, animal manure, composts, and reduced tillage (Poincelot, 1986).

Sustainable agriculture seeks to eliminate agriculture's consumption and pollution of limited resources so as to enhance the economic viability, environmental soundness, and social acceptance of agricultural production systems. It is defined as a site-specific, integrated system of plant and animal production practices that will:

produce adequate food, maintain environmental quality and the natural resource base, optimize the use of nonrenewable resources, utilize renewable resources, maintain optimal productivity of farming systems, and improve the quality of life for farmers and society. To achieve the goal of sustainable agriculture, efficient management methods must be adopted (Parr et al., 1992).

The basic element of sustainable agriculture is the conservation of energy, soil, and water. To conserve these resources, agricultural practices that will assure renewability and nonpollution of resources are essential. Such concepts do not imply the complete abolition of agricultural chemicals. More efficient methods undoubtedly will depend on using appropriate technology, a technology that is energy efficient, productive, profitable, and nonpolluting. However, a return to an agriculture free of pesticides and fertilizers would mean return to a less technological agriculture, one that would increase human labor and decrease agricultural productivity. Sustainable agriculturists will have to keep in mind maintenance of optimal productivity and minimal labor input along with resource conservation and environmental maintenance.

However, the level of sustainability of a particular management skill may not be known. Therefore, we need an acceptable method for evaluating the effects of the management method to judge which management skill is preferable for sustainable agriculture. Unfortunately, we have no scientifically acceptable method or procedure for quantifying and measuring soil quality. So the effects of management practices can not be evaluated accurately. Hence developing useful soil quality indices that characterize soil quality - i.e., the soil's capacity to produce crops at optimum levels, resist erosion, and reduce the impact of environmental stresses on plants - is important so we may monitor the progress of any corrective action.

The possible candidates for soil quality indices are the measurable soil attributes such as soil depth, water-holding capacity, bulk density, hydraulic conductivity, nutrient availability and retention capacity, organic matter, pH, and electrical conductivity. These attributes are primarily affected by degradation or rehabilitation processes. Soil quality is also indicated by infiltration, macropores, aggregate size and stability, aeration, and root penetration. Besides these properties, other possible indicators are plant health, the presence and type of microorganisms, and invertebrates, surface and groundwater quality, and plant roots. To quantify and evaluate changes in soil quality, a set of data must be identified.

One promising soil quality indicator is the plant root system (Smucker, 1994). Plant root growth is sensitive to environmental stresses and to changes in soil properties associated with soil management practices. Changes in plant root parameters such as length, surface area, or number may indicate the degradation or rehabilitation of soil. Soil depth that would restrict root growth and water movement may be measured by studying maximum root depth. Also the status of compaction can be learned by analyzing the root parameters, because generally an increase in bulk density and penetration resistance results in restricted root development. Plants in compacted soil have a denser and shallower root system than do plants growing in non-compacted soil

(Oussible et al., 1992). Because the plant root system is directly affected by environmental changes, roots can be meaningful indicators of sustainable agroecosystems. The maintenance or improvement of current soil conditions, and the reduction of environmental hazards, may be monitored by quantifying the development of the plant root system. 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).

Since root systems are the net result of time, space, and genetic responses to climatic and soil conditions, it would be most beneficial to incorporate multiple evaluations of root quantity, depth, etc. into the assessment of an agroecosystem. It seems logical that the length of roots within a soil volume at a given soil depth, at certain times during the season, would be an indicator for comparing the sustainability of multiple environments. This root parameter can be determined nondestructively by the minirhizotron and video recording methods, or destructively by sampling, washing, and analyzing the roots removed from the soil.

Nondestructive root observation techniques such as the minirhizontron, computer-assisted tomography (CAT), and nuclear magnetic resonance (NMR) imaging allow repeated observations of a particular plant root at specific dates, field locations, and soil depths. However, these techniques are very expensive and some of them are difficult to access. The initial cost of the minirhizotron, the most inexpensive technique, is about $20,000 ~ 100,000. Other restrictions on the use of these nondestructive root observation methods are the additional costs for maintenance and the fact that the minirhizotron tube perturbs natural root growth. These facts restrict the use of these nondestructive root observation methods.

One of the main drawbacks of destructive root observation techniques is the tremendous time required to measure the root parameters. Also, the techniques are labor intensive. Applying the image analysis technique can reduce the time and labor requirement for estimating plant root dynamics. 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: length, diameter, number of branches, density. The computer also provides accurate measurements of the plant root system.

Modeling of plant-soil systems requires accurate quantification of subterranean as well as aerial plant structures. Although a sizable research effort has contributed to solving the root quantification problem, more accurate, faster, and less expensive approaches are needed. Also further work is needed in formulating techniques to measure root length, root density, orientation, and branching on binary images, which have just two colors: black and white.

1.2 Sustainable Agriculture and Plant Roots

An information about root development is important for proper interrow cultivation and fertilizer practices (Stoskopf, 1981). Because of the high concentration of lateral roots in the upper soil zone, interrow cultivation would damage a significant number of crop roots. In some cases, the shallow cultivation results in minimum root damage. Knowledge of the root system also helps the proper use of fertilizer. Appropriate placing of fertilizer nutrients may be an effective way of fostering a desirable root morphology and increasing the efficiency of fertilizer use, thereby reducing the amount of fertilizer needed. Use of less chemical fertilizer would reduce pollution, energy demands, and costs.

Besides helping interrow cultivation and fertilizer practices, information about the root system contributes to proper irrigation. The quantity and frequency of water to be applied is determined by a large extent on the root development (Stoskopf, 1981). During early plant development, keeping the surface soil too moist may encourage a shallow rooting habit. The crop may later suffer from drought if not watered very frequently. Plants with deep roots generally survive drought better than those with shallow roots because more water is accessible deeper in the soil profile. Deep roots may be developed by a delay in applying irrigation water. A controlled irrigation may produce good results when water is applied at the appropriate time. Proper use of water is an economic necessity, because much of the water used for irrigation has consumed considerable energy to lift it, convey it, or pressurize it.

These roots provide essential information for effective soil management. Roots serve several other functions for the plant. They provide anchorage to keep the plant from being washed or blown away or from being toppled. Anchorage enables the shoot to emerge through soil crusts and helps roots force a path through a soil matrix. Roots conserve soil by providing organic matter, reinforce the soil matrix on a slope, shatter the compaction pan, and aggregate soil particles.

In order to satisfy the shoot's demand, roots take water and nutrients. These functions are complicated because roots have to deliver water and nutrients that have been absorbed concurrently from moist and partially dry soil, from deep and shallow soil layers, and from soil zones of different chemical, biological, and physical properties.

The size and morphology of a root system can have a profound effect on the extraction of nutrients from soil. For a given mass of root tissue, long, thin roots have a larger surface area than short thick roots and would be expected to explore the same soil volume more effectively.

Root extension is important to the absorption mechanism. Plant root systems are not static but constantly grow and expand into new areas of the soil, encountering more soil water as they extend (Troeh et al., 1991). Because water movement to roots by unsaturated flow occurs only over very short distances of a few millimeters maximum per day, root extension is important in aiding the plant's absorption of water, particularly when the soil root zone holds no gravitational water.

An considerable organic waste, such as roots, chaff, stems, and leaves, remains after crops are harvested (Poincelot, 1986). Among them, roots are the biggest contributor to crop residue. Crop can contribute to the control of soil erosion and sediment transport, the retention of nutrients through runoff reduction, and the maintenance of organic matter in the soil. In addition, they can improve several soil properties. For example, infiltration rates for water are increased. As a result water retention capacity, and soil aggregation are enhanced, and crusting of soil is reduced. A certain amount of organic matter is needed in agricultural soils. Organic matter has a buffering capacity that helps to prevent sudden pH changes resulting from the addition of fertilizers, lime, or sulfur. Organic matter also plays an important role in soil structure because it affects aeration and helps in water retention. Organic matter promotes the development of soil aggregates. Better root development results from improvements in aggregation (Poincelot, 1986).

Water infiltration is improved by organic matter at the surface, as drainage and water retention is improved by deeper organic matter. While organic matter is reduced through cultivation, drainage and water absorption decline, thereby increasing surface runoff and, consequently erosion of soil by water (Poincelot, 1986).

Organic matter contents and properties in the rhizosphere, which is the interfacial volume between plant roots and the bulk volume of the soil, differ from those of bulk soil. The root cap exudes a carbohydrate-rich gel, and root epidermal cells are mechanically ruptured as roots grow through the soil. These gels, root cell contents, and root exudates are chemically diverse, but serve as excellent substrates to support a great diversity of life and large numbers of microorganisms. These rhizosphere microorganisms include bacteria, fungi, viruses, yeast, etc. These microorganisms, along with the root, comprise a soil ecosystem that is vastly different from that outside the rhizosphere (Rendig and Taylor, 1989).

Besides aggregating the soil, the root itself may stabilize the soil. Mechanical reinforcement that stabilizes soil on slopes can be increased by plant roots (Waldron, 1977). Plant roots increase soil shearing resistance by both mechanical reinforcement and water removal by transpiration. Waldron (1977) states: "Root-reinforced soil may be analyzed as if it were a composite material in which fibers of relatively high tensile strength are embedded in a matrix of lower tensile strength. This is the basis of the engineering technique of reinforced earth in which true cohesion is imparted to soil by linear reinforcing elements." (p. 846). Tangential forces to the fibers produce different tensions along their length. Friction or bonding between the fibers and the surrounding matrix may carry these tangential forces. Therefore, the forces should differ according to the root numbers, root hairs, bending, and branching of roots.

Furthermore, many references show the pan-shattering abilities of various plants such as alfalfa and sweet clover. Elkins et al. (1977) indicates that bahiagrass roots will penetrate soil layers that mechanically impede crop roots. According to them, bahiagrass increased in pores greater than 1.0 mm in diameter. Thus, it increased cotton rooting densities to a depth of more than 60 cm. Peterson (quoted by Elkins et al.,

1977) has produced results showing that bahiagrass roots possess a fibrous sheath beneath the epidermis that gives the roots the additional penetrating ability of bahiagrass roots.

Finally, nitrogen fixation on legume roots is another important root function. Bacteria use energy produced by photosynthesis in the plant in order to convert inert atmospheric nitrogen into a fixed form usable by plants. As the legume plants grow, the symbiotic bacteria are able to use the inert nitrogen in the air and to multiply in the nodules on the root. The nitrogen becomes available to the legume plants and supports their nourishment and growth (Stoskopf, 1981). Utilizing this nitrogen would help reduce costs and energy demands and would remove a source of potential pollution because lesser chemical fertilizers would be required.

To use roots as a sustainability index, a great amount of information is needed about root systems. The relationship between the degree of sustainability and root system development is required. Unfortunately, not much information about root systems is known because the study of roots requires tremendous time and labor. A rapid method for extracting information from the root system is needed immediately.

1.3 Objectives

In the present research, computerized image analysis is used to measure the parameters of plant roots that will serve as a soil quality indicator. The overall goal of this work is to provide a general method for plant root observation. To achieve this goal, the two specific objectives formulated are:

1. To develop a rapid procedure for measuring plant root length *in situ,* and

2. To evaluate the use of an inexpensive image analysis system for measuring plant root length.

II. Review of Literature

2.1 Index of Soil Sustainability

Several terms describe gains and losses in soil quality: 'soil health index', 'indicators of sustainability', 'soil quality index', and 'soil productivity index'. All these terms were created to assist soil preservation or soil regeneration activities. An effective soil index can help to prevent environmental and economic disasters resulting from climatic catastrophe, human mismanagement, or misuse of agricultural chemicals. A soil health index will reveal those areas where soil is improving, and studying them and adapting those methods can improve soil quality worldwide (Haberern, 1992).

Parr et al. (1992) defined soil quality as the capability of a soil to produce safe and nutritious crops, and to enhance human and animal health, without impairing the natural resource base or harming the environment in a sustained manner over the long term. They explored the components of soil quality and suggested a mathematical relationship or model that could quantify the various attributes of soil quality. They also explained soil quality as a linkage between the strategy of alternative agriculture and the ultimate goal of sustainable agriculture.

The reason why a soil quality index is needed was described by Granatstein and Bezducek (1992). A soil quality index would help judge whether management and land use are having the desired results for productivity, environmental protection, and health. Farmers can evaluate the economic potential of new options and their impact on the soil resources with a soil quality index. A soil quality index would be useful for researchers and policymakers to evaluate policy decisions and measure progress. The indicator is also helpful to environmental and regulatory agencies to determine whether degraded soils have been restored satisfactorily. To develop a soil quality index, the authors considered a reference point, such as native soil, that could be used as a benchmark for comparison with differing management systems.

Gale et al. (1991) examined the usability of the soil productivity index (PI) model to estimate site quality for white spruce plantations. The sufficiency of available water capacity, the sufficiency of aeration, the sufficiency of bulk density, the sufficiency of pH , the sufficiency of percent slope, the sufficiency of climate (precipitation and evaporation potential), and the vertical root distributions were parameters for the PI determination.

Another productivity index (PI) that is based on the assumption that crop yield is a function of rooting depth was evaluated by Lindstrom et al. (1992). They evaluated the usability of the PI model as a method to predict soil productivity. They concluded that the PI model can be used to characterize soil productivity at specific sites if accurate field data are available.

The principal physical and chemical attributes that can serve as indicators of a change in soil quality under particular agroclimatic conditions were examined by Arshad and Coen (1992). Suggested indicators include soil depth to a root-restricting layer, available water-holding capacity, soil bulk density, penetration resistance, hydraulic conductivity, aggregate stability, organic matter, nutrient availability, retention capacity, pH, and, where appropriate, electrical conductivity and exchangeable sodium. The authors did not evaluate these indicators.

Physical, chemical, and biological soil characteristics were evaluated by Karlen et al. (1992) as criteria that can be used to evaluate human-induced effects on soil quality. Physical indicators included bulk density, porosity, structure, roughness, aggregate characteristics, and resistance to wind and water erosion. Chemical indicators that may be useful for evaluating soil quality were pH, cation and anion exchange capacities, total and available plant nutrients, salinity, and nutrient cycling or transformation rates. Biological indicators included microbial activity and natural processes of respiration, mineralization, and denitrification. The nutritional quality of plants in relation to the soil upon which they are grown was another category of soil quality indicators. Besides describing the indicators, the authors suggested some soil and crop management strategies that will sustain or improve soil quality, including reduced or conservation tillage practices, cover crops, and increasing temporal and spatial diversity by using different crop rotations.

Various types of soil microbiological parameters were assessed by Visser and Parkinson (1992) in monitoring soil quality. The authors classified soil microbiological criteria at three levels: population, community, ecosystem. Population level studies the dynamics - population densities, biomass, survival, and reproductive rates - of the important individual species. Community level studies estimate species diversity and frequency of occurrence of species. Ecosystem level studies focus on the processes involved in cycling organic matter (carbon) and nutrients (nitrogen), and on nutrient retention efficiencies. Visser and Parkinson suggested that ecosystem level approaches offer the best possibilities for rapidly assessing changes in soil quality.

2.2 Soil and Root Relationship

Key factors that influence root growth and distribution are soil water potential, soil temperature, oxygen stress, heavy metal stress, mechanical impedance and salinity stress, the presence of continuing supplies of water to maintain hydrostatic pressure in the elongating cells of the root, of metabolites for cell wall construction, and of growth hormones to loosen the bonds within the cell wall constituents. Root diameters are much more sensitive than are root elongation rates to changes in soil water potential. Decreases in either soil matrix or osmotic potentials cause roots to shrink in diameter. (Rendig and Taylor, 1989)

Excessive soil strength, sometimes called mechanical impedance or physical impedance, can severely affect the plant's ability to extend its root system into unexplored soil volumes. Excessive soil strength can arise as a result of increased soil bulk density, increased friction between soil particles, increased cohesion particles, or reduced soil water content. Compaction-collapsed soil macropores interfere with the movement of soil water, causing a halt to water movement and increasing erosion of soil by surface runoff. Several subsequent problems resulting from soil compaction are:

soils remain colder longer, causing delayed planting and slower seed germination; crop residue decomposes more slowly and losses of soil nitrogen from denitrification tend to increase; root development can be hindered, so that water and nutrient availability becomes a problem. Such conditions result in reduced crop yields.

Low soil temperatures generally cause slow growth of crop plants. In addition, low soil temperatures often reduce uptake of minerals and water, further reducing plant growth. High temperatures at the soil surface can cause death of young seedlings, especially those with very small diameters. Each species of plant has a minimum soil temperature below which no growth of roots will occur. Above that minimum temperature, root mass accumulates almost linearly with temperature to a maximum rate at an optimal temperature, and then decreases rapidly until a high temperature is reached where no accumulation occurs. Root diameters often decrease with increasing soil temperatures.

Respiratory energy is necessary for many plant processes and reactions to occur. For aerobic respiration to occur in roots, oxygen must be relatively free to move through air-filled spaces. If soil bulk density is great enough to cause partial blockage of air-filled pores, oxygen diffusion rates are reduced substantially. Oxygen stress sometimes occurs in conjunction with mechanical impedance in compacted soils but almost always is caused by excessive soil water content when it occurs in non-compacted soils. The symptoms of oxygen stress are relatively easy to diagnose when caused by a static water table. In that case, white, turgid roots will concentrate in a zone a few centimeters above the water table but will not grow into the water table very far (Rendig and Taylor, 1989).

The objective of Oussible et al.'s (1992) study was to evaluate the effect of subsurface compaction on the root and shoot growth, grain yield, and grain yield components of wheat. Field experiments were conducted in 1982 and 1983 on a Moroccan clay loam soil (typic Calcixerolls). Soil compaction was artificially created. The 0.10-m surface layer was removed from all plots with a road scraper. The exposed subsurface layer was then packed by making four passes over the plots with a 7.5-ton tractor. The removed soil was replaced and leveled. Control plots were tilled with a disk plow followed by two passes of a disk harrow. Both compacted and control plots were then rotovated for final seedbed preparation. The result of this compaction was a 12 to 23% decrease in grain yield and 9 to 20% decrease in straw yield. Both root growth and distribution were markedly changed as a result of subsurface compaction. Wheat plants in compacted plots had a denser, finer, and shallower root system than wheat plants in control plots. Plant height and leaf area and dry matter per shoot were unaffected. The decrease in shoot number might be attributed to a limitation in the amount of available soil N to the roots.

A root growth model was developed by Simmons and Pope (1988) to graphically simulate predicted root responses of yellow-poplar and sweetgum seedlings to changes in soil physical properties. Data for the model were collected in greenhouse and laboratory experiments. Newly germinated yellow-poplar and sweetgum seedlings were transplanted into pots containing silt loam soil compacted to bulk densities of 1.25,1.40, or 1.55 $Mg \cdot m^{-3}$ and grown under greenhouse conditions for 3 months. Minimum water potentials were maintained at -10 or -300 kPa. At harvest, root systems were excavated, divided into orders of lateral roots, and length, number, and branching frequency of each order were determined. Air-filled porosity and mechanical resistance were determined for soil samples equilibrated at the same bulk densities and water potentials as those used in the greenhouse study. Based on root and soil parameters, the model ROOTSIM graphically depicts the root distribution of each tree species at different levels of bulk density, mechanical resistance, and air-filled porosity. The model accurately predicts lateral root length and distribution for the range of soil properties used in the greenhouse study, but has not been validated for these or other soil conditions.

Bui and Box (1993) studied the effect of different corn root length densities, during vegetative and anthesis plant development stages, on interrill soil erosion. Roots were washed from the cores by a hyperpneumatic elutruator, and stained with a methyl violet solution. Root lengths were determined on a Delta-T analyzer (Delta-T Devices, Burwell, Cambridge, England), reported by Harris and Campbell (1989), which uses a line intersect method to estimate length. They concluded that high densities of live corn roots did not have a stabilizing effect against interrill soil erosion on well-shaped ridges in Cecil soil. They claimed that this result is consistent with the results of Barley (1954), who found that live corn roots decreased the permeability of sandy loam through pore blockage and soil compaction, as well as with those of Reid and Goss (1981), who reported that living corn roots decreased the stability of fresh soil aggregates.

Van Noordwijk (1991) summarized the quantitative data on specific root lengths (length/dry weight ratio) and root length densities in different layers of the soil from literature for various crops. According to him, in the past two decades root length and root surface area have replaced root dry weight as the root parameter of primary interest because root research has become more oriented to analysis of transport problems.

2.3 Root Observation Methods

2.3.1 Minirhizotrons

In situ root distribution and dynamics of Seyval grapevines were observed by McLean et al.(1992) using the minirhizotron and portable microcamera system. The root observation technique was used to evaluate root depth and density responses to fruit loading of vines growing under low soil-moisture conditions. McLean et al. inserted the transparent polybuterate tubes at 45-degree angles to the soil surface and parallel to the vine row before planting to prevent roots following the tube. The microvideo camera was inserted to the lowest root depth of the tube and the camera was raised up along the minirhizotron tube at 1.2 cm intervals every three seconds. The number of roots per three-second picture were counted on a monitor in the laboratory and recorded using software called “Minsort”. The root dynamics were monitored every two weeks for the two-year experiment. The minirhizotron system could measure root distributions through the soil profile successively.

Volkmar (1993) compared flexible- and rigid-walled minirhizotron techniques for root length density of 14- to 28-day-old bean and wheat plants in soil boxes at three different soil bulk densities. Roots were photographed and root length was quantified by the line intersect method. The results showed that bean root density decreased significantly with increasing soil bulk density but wheat root density was unaffected by soil bulk density. Also, the results showed that rigid-wall minirhizotron estimates of root counts were higher than those from the flexible-wall minirhizotron. The overestimates resulted from differences in the physical environment at the tube-soil interface.

Vos and Groenwold (1983) found a linear relationship between root length as seen through an endoscope on the interface of observation tube and soil and root density obtained from core samples in adjacent soil. They installed the observation tube at the soil containers when the containers were filled with a sandy soil. They took photographs at 5 cm distances along both upper faces of each observation tube. Negatives obtained by processing the photographs were projected on a screen which was covered with a grid-lined transparent sheet. Root lengths were calculated by the line intersect method. Also, they took core samples at 10 cm distances along the tubes.

The core samples were washed and the roots were spread on grid mesh. Root lengths were also calculated from the line intersect method. There was good deal of scatter between the two sets of data, but some correlation was evident between the two methods of studying root systems.

Meyer and Barrs (1985) described the technique of inserting clear acrylic tubes horizontally into large undisturbed and repacked soil cores. They counted the number of intersections between roots and scribed lines on the sides of the observation tubes using a fiberscope. They estimated root length density using Lang and Melhuish's equation ($L = 2n$, where L is the total length of roots within the unit cube and n is the number or roots intersecting one of the six faces of the cube). The estimated root length was compared with destructively determined values of root length density. They found a linear relationship between these two values.

Upchurch and Ritchie (1983) described a minirhizotron system for evaluating the rooting activity of field crops. They took root images by video camera and recorded on video recorder for later analysis. Minirhizotrons were in four orientations with respect to plant rows. Observations from minirhizotrons were converted to root length densities by counting sector intersections and using the equation : $RLD = Nd/Ad$, where RLD is root length densities, N the number of intersecting roots, d the outside tube diameter, and A the area of tube observed. The calculated root length densities were compared with those measured by soil core sampling. The results indicated a linear relationship between the two techniques. However, the results from several tubes had to be averaged before there was a satisfactory correlation with the bulk soil root length density, because of the variability of the results from individual minirhizotrons. According to the authors, the other problem in using minirhizotrons in root observation is that a significant portion of the roots that intersected the tube followed it for some distance.

Sanders and Brown (1978) described a technique for observing and photographing root development patterns within a soil profile using a fiber optic duodenoscope. Root lengths for soybeans were estimated by the duodenoscope and compared with root lengths measured by core sampling methods. Both methods used Newman's line intersection method to measure the root length. The correction factor 21.1 was multiplied by the number of root intersections determined from the duodenoscope photographs to correct for volume differences between the core and the photographs. The correction factor represents the ratio of the soil core volume to the volume represented by the photograph. The volume represented by the photograph was calculated by assuming the depth of view from the photograph as 0.3 cm. Their results showed that total root lengths from the two methods were in good agreement. For the 0 to 72 cm depth intervals, the measured root length by core methods was 56.36 cm, and the estimated root length by the scope was 52.84 cm. However, changing the value of the depth of view can cause a significant difference of estimation of the root length from photographs.

Bland and Dugas (1988) assessed three theoretical models to convert counts of roots at the walls of horizontally installed minirhizotrons to root length density (L). All models were in the form of L = C×(counts/area of tube wall). Each model used different theoretical values of C (Upchurch and Ritchie(1983): 1.0; Melhuish and Lang (1968): 2.0; and Upchurch (1985): 3.3 - quoted by Bland and Dugas, 1988). They reported that the coefficient 2.0 gave the best estimation of L when compared to measurements of L from roots washed from soil samples. However, the model was valid only on a certain plants.

Reicosky et al. (1970) compared three methods of estimating root length: the direct method, the inch counter method, and the line intercept method. The line intercept method had a tendency to overestimate the root length. However, the authors concluded that the line intercept method was as reliable as the direct count method.

Upchurch (1987) described the procedures for quantifying the intersections between roots and the minirhizotron tubes: (1) hand-drawn pictures of the intersecting roots, (2) measuring the root length in contact with observation tubes, (3) counting the number of roots growing against the observation tubes and crossing a given depth mark, and (4) counting the number of roots in contact with the observation tube within a given area of the tube. He also described the techniques for converting minirhizotron observations to root length: (1) deriving empirical relationships between root length density measured by another technique and root-tube intersections, (2) converting root length in contact with the tube to root length density by associating a particular soil volume with this length, and (3) deriving theoretical conversion factors. The empirical conversion method required a new conversion equation for each new crop and soil combination, and for each growth stage of the crop. This method required recalibration with each experiment. The length-volume relationship assumes that all roots within a specific distance of the wall of the tube are seen and that no roots can be seen beyond that distance. The distance ranged from 1 to 3 mm, depending on soil texture and specific experimental conditions. The theoretical conversion method was based on geometrical probability and the assumptions of random distribution and random growth direction of the roots. Lang and Melhuish (1970) presented a theoretically derived coefficient of 2 to relate the number of intersections per unit area on a plane surface of a unit cube to the root length density within the tube.

Buckland et al. (1993) proposed a method that allows a theoretical conversion of root counts in minirhizotrons to estimated root length density. They counted 'points of contact' at which a root intercepts the observation tube in a grid square. This method is different from others because most root length measurements use the line intersection method, which counts the number of intersections between roots and grid lines. To convert 'points of contact' to an estimate of root length density, the authors multiplied the diameter of the observation tube to half the number of 'points of contact'. Then they divided the calculated root length by the proportion of the observation volume which corresponds to the proportion of the surface of the tube that was counted. They compared the estimated root length density by their method to the root length density measured by core sampling. The results showed that their method agreed well with core sampling for wild cherry roots but not for roots of pasture species. The authors thought that the discrepancy in the results was caused by differences in the root size, topology, branching, and growing habit between wild cherry and pasture species. They also discovered that root counts were not uniformly distributed around the circumference of the observation tube.

McMichael et al. (1992) described transparent or 'glass wall' techniques for observing root growth in plants *in situ*. The techniques included rhizotrons, minirhizotrons, and slant tubes and slant boxes. According to their description slant tubes or boxes are usually for laboratory use. The difference between these two methods was in the container for the soil. These two methods were best suited for measurement of root elongation rates. They allowed rapid screening of a large number of plants for differences in rate of increase in rooting depth. Root biomass or total root length could be measured at the end of an experiment by washing out the roots in the tube. One disadvantage of using these techniques is that experiments must be conducted over a relatively short duration because the plants must be grown in a very limited soil volume. Temperature control had to be devised to prevent wide temperature fluctuations that may influence results. Rhizotrons were suitable to the study of changes in the size and activity of plant root systems. Minirhizotrons could be used to collect quantitative information regarding rooting depth, root length density, root morphological characteristics, root dynamics, and in pathological studies involving root systems. The main disadvantage of these methods was the high cost of installing such a facility and maintaining it. Another problem was an unrealistically high concentration of roots at the interface resulting from the air gaps at the glass-soil or acrylic-soil interface.

Taylor et al. (1990) described the advantages and disadvantages of using rhizotrons and minirhizotrons in root research. The main disadvantage of using rhizotrons is the expense; the advantage is that successive measurements must be taken on the same individual root or the visible part of the root system. On the other hand, the limitation of the minirhizotron system is the number of tubes required to accurately estimate rooting. Both methods involve different soil physical factors between the soil-tube or soil-glass wall interface and the bulk soil, so that it is difficult to estimate root parameters of the undisturbed soil matrix.

2.3.2 Core-Break

Melhuish and Lang (1968) derived a theoretical relationship between the number of root intersections on a single plane of known area and the probable total root length per unit volume of soil, using the principles of geometrical probability. The relationship between total root length and the number of intersections is $L_T = 2N_A$, where L_T = total root length/unit volume of soil(cm/cm^3), N_A = number of roots intersecting a plane of unit surface area (no./cm^2). The relationship is based on the assumption of random distribution of the roots within the soil volume being sampled.

Lang and Melhuish (1970) analyzed the relationship between the length of non-randomly distributed plant roots in soil and the number of intersections which the roots make with planes cut through the soil. They derived an equation that can estimate the total root length from finite counts of the intersections in three mutually perpendicular faces. The total root length in the soil cube was calculated by the equation: $L_T=(n_A + n_B + n_C)L_m$,where L_T is total root length, n_A, n_B ,and n_C are the number of intersections in three mutually perpendicular soil cube faces, and L_m is the mean secant of all lines passing through the cube. The L_m is read from the figure of the mean length of a secant in a cube as a function of the anisotropy of the angular distribution of secants.

Bennie et al. (1987) attempted to correlate the length of roots per unit volume of soil (L), and the average number of root intersections per unit breakage plane (N) for several different crops in the field. A linear regression equation of the form $L = bN$, was fitted to the data of each group of samples to find the relationship between the number of roots per unit soil area and the root length density. The regression coefficients ranged from 1.4 to 2.4. The taproot systems of dicotyledonous cotton and sunflower had a conversion factor of 2, indicating a random distribution of roots. Monocotyledonous sorghum's 1.5 indicated that orientation of the roots was more vertical than random. It seems difficult to apply Lang and Melhuish's equation directly to convert the number of roots per unit soil area to the total root length density. They suggested that different individuals count the same intersection numbers, to avoid subjective decisions and give more accurate results. This problem can be eliminated by using image processing.

Bland (1989) assessed the core-break method, in which the number of roots visible at a broken cross section of a soil core is used to estimate the root length density (RLD) of wheat, cotton, and sorghum on fine-textured Texas soils. He obtained a linear relationship between root counts and RLD for each crop on each day of sampling. However, the core-break method yielded a low-precision estimate of RLD from the mean of several core counts. A possible source of error was overlooking some roots by counts. The precision of this method can be increased by increasing the number of cores and increasing the diameter of the core. Also, using image processing in counting root numbers can eliminate the human error and increase the precision of the method.

Drew and Saker (1980) examined the relationship between root numbers observed on the faces of soil cores, and the length and weight of roots extracted from the same cores. They calculated regressions of the field estimate of root length (L), using Lang's equation, on either the root length or weight (X) of roots washed from the soil. The estimates of root length (L) were highly correlated with the length and weight of roots. However, the estimated length offered only an approximate guide to the absolute lengths of roots per unit soil volume at each depth, unless simultaneous calibration was undertaken on some of the soil cores by washing roots from the soil and making direct measurements.

Sources of error in estimating root length density from counts of roots at broken faces of a soil core were analyzed by Bland (1991). According to him, three sources of error affected accuracy of the method. The root length conversion equation is based on the assumption that root growth is equally probable at every angle, but distributions of root angle can be non-isotropic. The second source of error was 'systematic gradients' of root length density in the soil. The third depends on how roots are dispersed in a particular core.

Baldwin et al. (1971) developed a method for measuring root length and distribution for plants, which uses the same basic principle as that of Melhuish and Lang. They used an autoradiographic method for counting the number of root intersections. The isotope ^{32}P was injected into leaves to stain the roots. The authors derived a function for calculating the true root length of non-random oriented root systems from spot counts on three mutually perpendicular autoradiographs. The estimated root lengths were compared with the lengths of roots measured by image analyzing computer (Quantimet). The estimated root lengths agreed well with the length of roots measured by the Quantimet.

Fusseder (1983) used the autoradiographic method for studying the distribution of the corn root system. They labeled the living part of the root system by radioisotope. After 24 hours the rooted soil was deep-frozen with liquid N_2 and removed from the pot. The soil sample was sectioned into thin slices by a diamond cutting blade. Autoradiographs were taken on x-ray film for the surfaces of these slices. They placed a grid on the autoradiograms and counted the numbers of spots, that is, the cut root surfaces present in the soil cross section, in each square. They interpreted the distribution pattern of the roots by the average distance between the centers of the spots and the V/M ratio, where M is the average of the number of spots per square and V the variance of these mean values. They also calculated the root density per soil segment by the Melhuish and Lang equation (1968), $L_v = 2N$, where N is the arithmetic mean of the number of spots per cut plane and L_v the length of root (cm) per unit volume of soil. They checked this root length measurement method by investigating the root system of comparable plants with the intersection method of Newman, as modified by Tennant. They claimed that the two methods were in full agreement. Their results showed that the roots usually had formed more or less dense aggregations. However, this method has disadvantages. High roots concentration in a soil layer prevents single roots from being distinguished separately from the large black dots on the film. The radioactivity of the fine roots was not always strong enough to stain the film.

2.3.3 NMR

The NMR Imaging technique was used by Matyac et al. (1989) to observe the root galls. The NMR technique was evaluated for observing the development of galls caused by *Meloidogyne incognita* without removing the roots from the potting medium or disturbing the rhizosphere. NMR imaging could acquire root images without removing the roots from the potting medium. If cost and accessibility are not a concern, NMR imaging can serve as a noninvasive method for observing roots.

2.3.4 Comparison Between Root Observation Methods

Bohm et al. (1977) compared five methods for characterizing soybean rooting density and development. The five methods were soil water-depletion, framed-monolith, soil core, mini-rhizotron, and trench-profile. Each method had its own advantages and disadvantages. The soil water-depletion method was fast, needed only simple equipment, and provided satisfactory estimates of rooting depth but did not provide reliable estimates of rooting density. The framed-monolith method provided quantitative estimates of rooting density with depth but was very time consuming. The core sampling method was fast and could be used to estimate rooting density. The mini-rhizotron method was fast but did not provide accurate rooting density data. The trench-profile method was rapid and provided semiquantitative estimates of rooting density with depth and distance from the row.

Bragg et al. (1983) compared four methods of measuring root distribution in a spring oat crop. The methods were minirhizotrons installed vertically, minirhizotrons installed at an angle of 45°, core-break root counts, and direct measurement of roots washed from soil. Both minirhizotron methods underestimated root density in upper soil layers because roots are channeled down the glass-soil interface. Root densities estimated by the angled minirhizotron method and by the core break method were highly correlated with root length density of washed roots measured by the modified line intersection method.

Taylor et al. (1991) compared several root investigation techniques. These techniques included a simple spade, the core-break, minirhizontrons, computer-assisted tomography, neutron radiography, and nuclear magnetic resonance imaging. According to their comparison, a technique with a spade cost very little; however, this method destroyed the roots being observed. The core-break method could quantify root development under various soil conditions rapidly. However, it sampled only a limited part of the root system and destroyed the roots being studied. The mini-rhizotron technique required the insertion of an access tube, marked at predetermined depths, into the soil but then repeated observations could be made of roots at the soil-tube interface. CAT scanners were very expensive and there was a problem of equipment accessibility because CAT scanners usually were located in major medical facilities. The Neutron radiography technique readily provided good images of roots in soil up to soil thicknesses of about 5 cm. However, safety for the investigators and accessibility to a neutron source were the principal limitations to using this technique. NMR imaging was nondestructive and non-invasive but expensive.

Samson and Sinclair (1994) compared the minirhizotron and the soil core techniques to measure maize root length density obtained under field conditions. They found a substantial disagreement between the two techniques in the top 30 cm of soil. The minirhizotron technique consistently underestimated root length density at the soil surface layers. The relationship between the minirhizotron and the soil core results varied even when the observations from the soil surface layer were ignored. They concluded that directly translating minirhizotron observations into a root length density using a correlation with the root length density of the soil core technique was doubtful. Their results showed that the root length density of maize for the top 30 cm depth was usually 2 to 5 cm/cm^3, while at greater depths root length density was commonly less than 1 cm/cm^3.

2.4 Application of Image Analysis to Root Observation

Smucker et al. (1987) described the process quantification of roots from video recordings of washed roots and roots growing against minirhizotron tubes *in situ* using image analysis. In this procedure, images taken from video recordings were preprocessed to accurately separate the roots from the background. In the preprocessing, 256 video frames were averaged. Several filterings were performed to eliminate noises. Thresholding was used to discriminate roots from soil background. The binary root images produced from the preprocessing stages were thinned down to a single pixel line or skeleton. Before thinning, the edges were smoothed, isolated pixels were deleted and broken edges were connected. The root width information was encoded on every point along the root center line. Also, root branchs and intersections were analyzed by separating each segment from the other root segments that intersected. Each thinned root image, root width image, and the branch and intersection image were contained in the separated image frames. Three image frames were used to extract root information. Roots lengths from both the image analyzer and the line intersect were compared for washed roots. Manual and digital analyses were compared for minirhizotron images of corn roots. Line intercept estimates of length gave greater values than image analysis estimates. However, image analysis estimates were very similar to manual measurements for images taken from field minirhizotrons.

Ferguson and Smucker (1989) used the minirhizotron technique with image analysis to observe roots in soil. Horizontal marks had been scored on the external surfaces of minirhizotrons for the purpose of estimating root lengths at given soil depths. During the successive image acquisition, the exact repositioning of the camera was impossible, and there was little opportunity for electronically locating and identifying specific root images by automated image analysis. A registration handle and the video recording of digital histogram messages denoting the field location, maximum rooting depth, and camera position within the soil profile were developed to locate and identify specific images for a specific sample spot. This approach enabled the field operator to repeatedly record specific images of the root system at multiple depths.

Girardin et al. (1991) compared three image acquiring methods, and also compared three root measurement methods. The image acquiring methods were rhizoscope, video camera, and endoscope; the root measurement methods included manual, Newman's method, and image analysis. They collected root image data from minirhizotrons placed in a maize field. They compared each technique's root length density (cm of root per cm^2 of tube area). To estimate root length from a photograph from video recordings, they used the Newman method. Because of the differences in the original picture size for all techniques, the results were expressed in root length density rather than total root length. There was no significant difference between each technique of recording and each method of measurement. However, they found that the rhizoscope has the advantage for automatic analysis.

A digital picture processing technique for evaluating plant root dynamics was used by Casarin et al. (1991). The researchers used the rhizoscope for getting images of roots *in situ*. The images of roots in contact with the outside wall of the tube were stored and processed on a computer. It made possible the processing and automated measuring of all important root properties, such as length, diameter, and branching. The authors found a number of difficulties arose when processing the root images obtained *in situ*: a) superposition of images from the same tube at different dates; b) electronic noise; c) ground noises such as droplets of condensation, and scratches on the tube; and d) variable luminance along the same root and according to diameter. To overcome these difficulties, several image enhancement techniques were used. Linear convolution was used to increase contrast for small and medium diameter roots. Depending on the coefficients and the size of the regional matrix, this technique also provided a preliminary noise reduction on uniform luminosity areas of ground. Morphological filters were used to reduce noise without a loss of definition. Two morphological techniques were adopted for diameter classification: 1) The distance function: the overall image was reduced to its basic skeleton; then the pixels common to the original image and its dilated skeleton were counted. 2) Step by step erosion: The corresponding structured elements were used only to erode the image in one direction for each transformation. They evaluated automated measuring of the total length of the roots by two other manual measuring techniques.

A root observation method using computerized image analysis was described by Commins et al. (1991). Root images were obtained from planar faces cut from a resin-fixed pot. Soil blocks were cut from the resin-impregnated soil sample pot to expose a planar face. The purpose of using rapid-curing epoxy resin was to fix the soil sample to allow the block to be cut while maintaining soil structure and root position within that soil structure. Autofluorescence using long-wave ultraviolet light and appropriate filters was used to contrast roots from the soil matrix. Captured images were segmented by simple bi-partitioning. Most results of the resin impregnation were unsatisfactory. The resin infiltration was hindered by soil moisture. The results for the root measurement parameters showed that roots appeared to be more concentrated in the upper portion of the section, and that surface area decreased with depth in accordance with the decreased root density. The problem with this method is the complicated procedure of resin impregnation. This procedure takes a long time, during which roots could dry out, thus changing root diameter significantly.

Cunningham et al. (1989) compared root length estimated by the modified line intersect method with root length estimated using a video image analyzer. A Delta-T area meter operating in the length measurement mode was used to count the number of intersections between the camera scan line and the edge of objects on the basis of sharp contrast between the background and the object. The meter readings were converted to lengths, using $L=(A+B)\pi/4$ as a calibration method (A and B = cumulative sums of projections in the field of view, L = estimate of the length). The modified line intersect method was performed manually. The root length was calculated using Tennant's formula $R=(11/14) \times N \times G$ (R = root length, N = number of intersects, G = grid size). To provide sufficient contrast for reliable determination of length with the image analyzer, photocopied images were used. In an experiment in which the lengths of a 0.9 mm diameter wire network were measured, the video image analyzer estimated the length by nearly the same as the actual length of a wire network. However, in an experiment estimating lengths of white oak roots, the video system underestimated the length of the roots by nearly 50%. The underestimate was caused by the meter's inability to distinguish some fine root images and loss of detection by root crowding in the field of view. Although the video system underestimated the root lengths, the video method was found to be linearly correlated ($R^2 = 0.92$) with the modified line intersect method.

Voorhees et al. (1980) proposed a method for determining root length using computer controlled scanning and digitizing of photographic images of roots. They used photography equipment to take root images. The equipment consisted of a camera mounted above a translucent plastic pan that was backlighted with fluorescent bulbs.

The negative root photographs, providing a contrast between "white" root against a "black" background, were scanned by a computer-programmed electronic scanning and digitizing system (Computer Eye Model 108, Spatial Data System, Inc., Goleta, Calif.). The system consisted of a light table, a scanner television camera, a digitizer, a video display, a digital computer, and a line printer. The television camera could scan up to 512 vertical lines; each scan line consisted of 480 digitized points. Each brightness value change, from peak to valley, along the scan line was counted as an intersection if the peak brightness value was greater than the preset zero base line. The number of intersections was inserted in Newman's formula to calculate the root length. The authors used photographic images of white string to calibrate the method. However, they did not show the data or the experimental procedure for real plant root length measurements.

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. Mathematical corrections were derived for overlapping roots in the sample and for limitations in system resolution. Roots were scanned in two directions to minimize errors due to nonrandom orientation. The calibration and roots overlap correction procedures were 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. Several tests were made using wheat roots. Coefficients of variation for repeated measurements on a single, undisturbed wheat root sample were around 0.5%.

The Fine Root Extraction Device(FRED) was developed by Pallant et al. (1993). The authors claim the device is inexpensive (less than US $50) and efficiently recaptures marked roots introduced to the FRED. The device was comprised of a plastic bucket, tygon tubes, airstones, air pumps, and support rods. Live roots floated to the top of the bucket when the gentle effervescence was generated. The roots were captured by several sweeps of sieve through the water. The lengths of roots were measured by digital image analysis systems using the line intersect method.

A flatbed scanner and a microcomputer were used by Pan and Bolton (1991) to measure root lengths. Algorithms for estimating root area, length, and mean root width were described. In this paper root area was estimated by summing the number of pixels representing a single scale or group of gray scales. Length and width were calculated as a function of the area, the number of edge pixels, and the number of pixels on diagonal edges. At higher root densities, lengths were underestimated and widths were overestimated due to the increased frequency of adjoining edges.

Smit et al. (1994) used a three-dimensional (3D) scanner for measuring the length of washed root samples that were obtained by auger from grassland vegetation. The three-dimensional scanner was used to overcome the problem of a low resolution of using video camera. The scanner enabled very thin roots (diameter 100 μm) to be segmented from the background in the gray-value image. Root image was skeletonized and the total length of the roots was calculated by multiplying the number of skeleton pixels of root by a correction factor. The correction factor was adopted to compensate for the difference of the lengths between randomly angled line segments to a horizontal line. This method was evaluated by comparison with the modified Newman's Line Intersect Method. The lengths measured with image analysis and with the line-intersect method were highly correlated (r^2=96.92%). However, this correlation was only valid on an area of 10×15 cm image. The authors said that when the root length was counted on a small area (6×6 cm), many roots were not counted.

Lebowitz (1988) described a new method for measuring the length and diameter of small root samples based on digital image analysis. The new method was called the Direct Discrete method. The method thresholded gray scale root image to binary root image according to the differences in brightness by examining the gray scale value frequency distribution of a digitized root image. Then the root image was reduced to a roots' skeleton that represent the roots' medial axis by a thinning algorithm. Finally, root length was computed as a function of the number of vertically, horizontally and diagonally adjacent pixels in the skeleton. The method based on a thinning algorithm was more accurate and precise than the Line Intersect method.

Shuman et al. (1993) compared the video image analyzer method to a hand method of measuring root lengths. The hand method used a ruler scaled in millimeters, and the video image analyzer method used a video camera, Delta-T devices and a meter (Burwell, Cambridge, England), and a video monitor. Root samples were taken from a series of four nutrient solution experiments concerning aluminum toxicity to sorghum seedlings. They used relative values to compare the instrument-measured and hand-measured root lengths. They claimed that normalized instrumental root length data were well correlated with hand measured root length data ($r^2 > 0.96$).

Ottman and Timm (1984) combined the Image Analyzing Computer with a staining technique by differentially staining cultured onion root tissue based on viability. Onion roots were stained with trypan blue and photographed. The processed photographic negatives were analyzed by the Image Analyzing Computer to find root length and surface. The authors gave no detailed information about the Imaging System.

Smika and Klute (1982) determined the surface area of washed roots with the Image Analyzer. Roots were photographed, and the developed film was scanned with an Image Analyzer. This value was multiplied by π, assuming the roots were cylindrical, to give the total surface area of the roots.

Yanuka and Elrick (1985) proposed a procedure for microcomputer-based digitization of images to estimate root length and width. The principle of root length estimation was based on the line intersect method. They evaluated the method by comparison of the actual and the estimated lengths of strings.

Heeraman et al. (1993) adapted a color composite technique to detect barley root dynamics from sequential minirhizotron images. Roots were discriminated from the soil matrix by assigning a separate primary color. Also, changes in root appearance and disappearance were detected by overlaying images which had a separate primary color to selected growth stages. They used a linear contrast stretch to enhance the image. To express the dynamics of barley roots, relative percentage areas occupied by the root and the soil matrix, respectively, within a 2.4 cm^2 viewing area were used.

In a study estimating tree root biomass, Ruark and Bockheim (1988) could discriminate tree roots from soil and rocks by using digital image analysis. They established a large trench to take a root image. A 1 % solution of Rhodamine WT dye, an anionic, maroon-colored dye commonly used in water-tracing (WT) studies, was sprayed to stain roots for producing a high contrast between roots and soil. A 35 mm camera, loaded with color infrared slide film, equipped with a 50 mm lens, and employed with a dark yellow filter, was used to take a picture. By this method, they could show that the total cross-sectional area of aspen roots visible in the soil profile and the biomass of aspen roots in the associated soil monolith were correlated ($r=0.64$).

2.5 Application of Image Analysis to Measurement of Plants and Plant Parts

van de Vooren (1994) used the image analysis method to measure the length and width of the pods and the length of the beaks of French beans. He used the distance transform and the distance skeleton method for measurement of length and width. Compared to manual measurement, the computer vision measurement was more than 3 times faster.

Tagliavini et. al. (1993) used the digital image analysis approach to estimate total surface area and average diameter of roots in experiments with peach seedlings grown in solution culture. They estimated the root surface area by 1) determining a gray-value threshold corresponding to the fine roots displayed on the video image; 2) producing a binary image; 3) determining the total number of pixels occupied by roots; 4) determining the proportion of the total pixel matrix occupied by a root image and transforming this to a two-dimensional root surface area; and 5) multiplying the two-dimensional root surface area by π to obtain total root surface area.

2.6 Root Parameters and Their Measurement

2.6.1 General

Root growth and distribution are commonly expressed by number, weight, surface area, volume, diameter, length, and the number of root tips (Bohm, 1979). Several approaches can be used to express root data in experiments. The parameters by which the root data is best expressed should be selected before starting an experiment. General criteria for determination of the root parameters are the research purpose, and the time and labor needed. For interpreting root data, it is often better to measure more than one parameter.

2.6.2 Root Number

Root numbers can be used to express the rooting density in a soil profile. Root number is not an ideal parameter because long and short roots are regarded and counted as equal units. However, high correlation with other root parameters is possible, especially root length. Also root number alone can provide a good estimate of the relative extent of rooting. This parameter may be utilized in studies employing all techniques of observation: excavating, profile wall method, core break method, and rhizotron method. The number of roots exposed along the face of an excavation or on the surface of a soil core segment is easily counted.

2.6.3 Root Weight

Root weight is used for studies of root growth in response to environment. It well characterizes the total mass of roots in a soil, when the productivity of underground terrestrial vegetation is to be determined, and the contribution of the roots for the amount of humus in the soil is to be studied. Root dry weight is used as the criterion for evaluation. Root weight also serves as a fundamental measure of photosynthate storage in a plant. However, only a small fraction of the total weight is represented by the amount of fine roots that can be the most active part of the root system. Consequently, root weight cannot be used as a parameter for characterizing the root activity, such as water and nutrient uptake. Generally, dry weight is preferred to get information concerning the growth and function of the roots. Dry weight is determined by drying in an oven at 105°C for about 10 to 20 h depending on the amount of roots. To distinguish the weight of contaminating soil particles on the washed roots, it is recommended that the dried roots be put into a muffle furnace at about 650°C. Then, the ash-free organic root matter, the difference between the root dry weight, including the soil particles adhering at the roots, and the weight of the ash residue, are determined.

2.6.4 Root Surface Area

Root surface area seems a good index of the capability of water uptake or nutrient uptake of a plant. Root surface area is determined directly by multiplying the average diameter of a large number of individual roots to the total root length per sample. Photoelectric devices can also be used for estimating root surface areas. The projected root area is measured by using photocell and galvanometer. Then the projected area is converted into the total root surface by multiplying by π, assuming that the measured roots are circular in cross-section. Determining the root surface by dipping freshly washed roots into a dye solution and measuring the amount of dye adsorbed on the roots is another possible method.

2.6.5 Root Volume

Root volume measurements can be related to the total absorbing surface area of the cortex and to the capacity of the root system to store nutrients. Only a few root researchers have used root volume as a parameter because root volume measurements from species with few large roots can be equal to species with large amounts of small fibrous roots. So root volume is used to supplement other parameters. Root volume can be calculated by multiplying the average root cross section area and the total root length. In fact, the water displacement technique, which uses a special container with an overflow spout, is to be preferred to measure root volume.

2.6.6 Root Diameter

Root diameter is used to calculate root surface or root volume, and it can be used to give information on the relationship between the pore size in a soil and the potential of root penetration. Root diameter can be measured on freshly washed root samples with the aid of a microscope fitted with an ocular micrometer, a micrometer or calipers. The diameter of roots can shrink in dry conditions, and such potential shrinkage must be taken into account during the measurements. Sometimes roots are classified into several classes according to their diameters for convenience (<0.5 mm very fine, 0.5-2 mm fine, 2-5 mm small, 5-10 mm medium, 10-20 mm large, >20 mm very large). However, this classification should be regarded as arbitrary because there is no evidence that shows a certain class of root diameter is related to any particular kind of root function (Bohm, 1979).

2.6.7 Root Tips

Root tips are a better parameter than root weight but are not well adapted for routine studies because the counting procedure is very time-consuming.

2.6.8 Shoot-Root Ratio

Shoot-root ratio is the common parameter for evaluating the relations between above- and below-ground growth of plants. Information about shoot-root relations is important for analyzing or simulating the growth pattern of whole plants and for estimating primary productivity of ecosystems. The ratio is a measure based on the dry weight of the shoots and roots of the plants.

2.6.9 Root Length

Root length is one of the best parameters for calculations 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 study is the possibility for rapid determination. These reasons make root length the preferred measure in root studies.

Root length can be measured directly by using ruler or ruled graph paper. However, these direct measurements are tedious and time consuming, so are not recommended as a routine method in ecological research.

2.6.9.1 Line Intersection Methods

The time required to measure root length is reduced by using the line intersection method. This method calculates root length rapidly by counting the intersections between roots and a regular pattern of lines. According to Newman's theory, root length can be estimated by the equation

$$R = \pi AN/2H \tag{1}$$

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. Usually the root samples are spread out on a flat surface, such as a shallow dish made of transparent plastic or glass under which is placed a grid. Then the number of intersections between the roots and the random straight lines is counted.

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. The size of grid depends on the amount of roots. A 1 cm grid is used for small root samples with total length below 1 m, a 2 cm grid is used for larger samples to about 5 m, and a 5 cm grid is recommended for total root lengths up to 15 m. Although an excessive number of intersections to be counted in one root sample can cause error from fatigue of the observer, a number below 50 of the counted number of intersections can decrease the accuracy of the results (Bohm, 1979).

2.6.9.2 Automated Root Length Measurements

Root length measurements can be done more rapidly 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 (Cunningham et al., 1989; 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.

The main drawback of the video image analyzer system that utilizes line intersection is that the root length measured by the device is affected by scan direction. And the number of parameters that can be measured by the device is limited.

A digital picture processing technique developed by Casarin et al. (1991) for evaluating plant root dynamics could overcome the limit that the device utilizing the line intersection principle has. In this method, the overall image is reduced to its basic skeleton; then the pixels of its eroded skeleton are counted. This method eliminates additional scanning for different directions, and makes possible the measuring of other root properties, such as diameter and branching.

Other researchers (Lebowitz, 1988; Smit et al., 1994) also used a skeletonizing algorithm in their root parameter measuring method based on digital image analysis.

The methods that are based on the skeletonizing algorithm were more accurate and precise than the line intersect method.

III. Materials and Methods

3.1 Root Parameter Selected for This Study

Root length and surface area seem the best parameters for estimating water or nutrient uptake. Physiologically, these two parameters appear to influence the plant functions the most.

Between the two parameters, root length can be rapidly measured *in situ* by using the digital image analysis technique. The reduced time for measuring this parameter can help us collect more data for establishing the relationship between the root parameters and the soil on which the plants are growing. Finally, we can get enough information for developing a 'soil quality index'.

3.2 Image Analysis System

3.2.1 General

To help understanding how root length can be measured by image analysis, basic knowledge about image processing is needed. An image is a visual representation of an object or group of objects. Image processing manipulates information within an image to make it more useful. Digital image processing is a specific type of image processing performed with a computer.

In order to process an image with a computer, the image must be converted into numeric form. This process is known as image digitization. The digitization process divides an image into a horizontal grid, or array, of very small regions called "pixels." In the computer, the image is represented by this digital grid, or bitmap. Each pixel in the bitmap is identified by its position in the grid, as referenced by its row (x) and column (y) number. By convention, pixels are referenced from the upper-left position of the bitmap, which is considered position (0,0).

When digitizing a gray scale image, each pixel in the image is individually sampled, and its brightness is measured and quantified. This measurement results in a value for the pixel, usually an integer, which represents the brightness or darkness of the image at that point. This value is stored in the corresponding pixel of the computer's image bitmap. The values are manipulated to provide more useful information.

3.2.2 Image Acquisition

Root images should be converted to digital form so that information can be manipulated and drawn out. There are essentially two methods for acquiring image data for processing: recording devices that are specifically designed to produce digital image data from scenes or photographs, and devices that produce digital image data from an analog source.

Images are generally recorded by a vidicon tube or charge coupled device (CCD). The sensor converts the light falling on it into a small voltage or current. The sensor output is buffered, amplified, and converted into a voltage suitable for sampling. This voltage is converted into a numeric value by an analogue-to-digital converter(ADC). This converter compares the incoming voltage with a set of standard voltages. The result of each comparison is a single bit value indicating whether the incoming voltage is higher or lower than the reference. The timing and address logic ensures that the incoming signal is sampled at the appropriate time and that the resulting binary value is stored at the correct position in the array, which represents the image. If the system is digitizing color information, 3 values are returned from the sensor for each pixel. In RGB color system, these are the red, the green, and the blue components of the color at that pixel.

CCD-based cameras are common image input devices. They are usually small and very light. The detector chip consists mainly of a large number of small cells, one for each pixel. A charge builds up when these cells are illuminated. The brighter the light, the more charge accumulates. After an appropriate sampling period, the charge must be read out from the cells and converted into a voltage. Another input device, a scanner, differs from a conventional camera by using a sensor that acquires only one row of images at a time. To capture an entire image the sensor must be swept mechanically across the field of view.

Another method to acquire images is to use a digitization system which is not part of the camera itself. Often the system will be mounted on an adapter card that can be used in a personal computer. Such an adapter is commonly known as a frame grabber. The adapter produces the digital values of the signal and ensures that the values are placed in the correct entries in an image array. A typical frame grabber digitizes the incoming signal at 512 points in time equally spaced throughout the duration of the row. In this study, a black-and-white CCD-based video camera was used for taking washed roots in the laboratory, and a color television camera for recording root images in the field. The field-taken images were digitized with frame grabber. During the laboratory experiments, the images from a black-and-white video camera were digitized with a video frame grabber-digitizer board (Data Translation, DT 3851). Then the digitized images were stored in a computer hard drive for later use. In the field experiments, the root images were taken by television camera and stored on a videotape in analog form. The analog image data were digitized with the frame grabber-digitizer board and then analyzed in the laboratory.

The video signal from 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.

3.2.3 Image Display

The cathode ray tube (CRT) is the device used for the display of image data. The technology involved is essentially the same as that used in domestic television sets. In fact, image display monitors have higher resolution and better geometric accuracy. There are additional electronics in the display controller to store the image data and to convert the digital data into a signal that the monitor can use.

To display an image, the following processes are carried out by the display controller. First, each pixel of the image is retrieved in turn from the storage area. Retrieval starts with the pixel at the image origin and proceeds along each row of the image. This process is called serialization. The timing and address logic ensures that pixels are retrieved in the right order and at a rate suitable for the display monitor, so that the position of the beam, which scans across the CRT to produce the image is maintained, and each pixel's brightness or color appears at the appropriate position on the screen. As each pixel is retrieved, it is presented to a look-up table (or palette). The LUT, a high-speed table look-up device, contains one table entry for each of the possible pixel values. For example, if a pixel value of 52 is retrieved, the LUT will return a table entry of 52. The contents of this table entry are used to determine how to specify the brightness of the spot on the CRT corresponding to the current pixel. Finally, the digital values from the LUT are converted to analogue voltages by a digital-to-analogue converter before being used to drive the CRT. This converter produces a voltage proportional to the digital value applied to it.

A 15 in diagonal NEC MultiSync 4FGE color monitor was used to display the control information of the operational software and the root images. The 15 in monitor has 0.28 mm dot pitch, and can display up to 1024 pixels horizontal by 768 pixels vertical with 76 Hz refresh rate.

3.2.4 Image Enhancement

An image requires some form of enhancement to improve its appearance to increase the ability to extract data from it. There are three basic ways to enhance an image: modifying the intensity value, applying a spatial filter, and manipulating the image frequencies.

3.2.4.1 Modifying the Intensity Value

One way to enhance an image is to change intensity values. For example, if an image is very dark overall, it can be brightened by boosting all the intensity values by a certain amount.

The common intensity manipulation tools are brightness, contrast, thresholding, histograms, and gamma correction. Brightness is a term used to describe the overall amount of light in an image. Contrast is a term used to denote the degree of difference between the brightest and the darkest components in an image. An image has poor contrast if it contains only harsh black and white transitions, or contains pixel values within a narrow range. An image has good contrast if it is composed of a wide range of brightness values from black to white. During a contrast operation, each pixel value is scaled by a contrast value, which serves to redistribute the intensities over a wider or narrower range. Increasing the contrast spreads the pixel values across a wider range, while decreasing contrast squeezes the values into a narrower range.

Gamma correction is a specialized form of contrast enhancement that is designed to enhance contrast in the very dark or very light areas of an image. This control is achieved by changing the midtone values, particularly those at the low end, without affecting the highlight and darkest points. Gamma correction can be used to improve the appearance of an image, or to compensate for differences in the way different input and output devices respond to an image. The gamma control modifies an image by applying standard, nonlinear gamma curves to the intensity value. A gamma value of 1 has no effect on the image. An increase in the gamma value will generally lighten an image and increase the contrast in its darker areas. A decrease in the gamma value will generally darken the image and emphasize contrast in the lighter areas.

Thresholding reduces an image to just two colors: Black and white. This is done by specifying a range of intensities to be emphasized, setting them to white, and converting all others to black. Thresholding is often used to segment an image in order to extract its important features.

A histogram measures and illustrates the brightness and contrast characteristics of an image in graph form. A histogram shows any brightness/contrast deficiencies that may exist in an image. Images with low contrast have histograms that are clustered around a very narrow portion of the color range. The position of the cluster indicates whether the image is too dark, too light, or simply too gray. The histogram can be redistributed by Histogram Equalization to enhance contrast and dynamic range nonlinearly.

3.2.4.2 Spatial Filtering

Filtering operations reduce or increase the rate of change that occurs in the intensity transitions within an image. Areas in which there are sudden or rapid changes in intensity appear as sharp edges in an image. Areas where there are gradual changes produce soft edges. Filtering acts to detect and modify the rate of change at these edges. It can increase the intensity differences in a soft edge to smooth and soften it.

Filtering can also be used to reduce image noise. Filtering is achieved by modifying a pixel's value based on surrounding pixel values. For example, blurring is accomplished by averaging all of the pixel values in a specified region, and replacing the center pixel with the averaged value. This process reduces the variation among neighboring pixels, and visually softens the image. A sharp edge would be softened with intervening levels of gray.

Filtering techniques are divided into two categories: convolution filters (linear filters) and nonconvolution (nonlinear) filters. Both techniques accomplish their results by examining a square region of image pixels, typically 3×3, 5×5, or 7×7 pixels in size.

3.2.4.2.1 Convolution Filters

Convolution filters process image neighborhoods by multiplying the values within a neighborhood by a matrix of filtering coefficients (integer values). This matrix is called a "kernel." It is the same size as the neighborhood that it is being applied to. The results of this multiplication are summed and divided by the sum of the filter kernel. The result replaces the center pixel in the image neighborhood. The convolution process always uses a neighborhood's original (unfiltered) pixel values as input.

The following convolution filters are commonly used. The Low-Pass filter blurs an image by modifying a pixel value to be more like its neighbors. This modification eliminates harsh edges by reducing the intensity differences between adjacent pixels.

The Low-Pass filter can be used to blur an image, eliminate detail in preparation for object segmentation, or eliminate random image noise.

The Hi-Pass filter accentuates intensity changes in an image by modifying a pixel's value to exaggerate its intensity difference from its neighbors. This filter produces an image with harsh intensity transitions, and generally results in an image with only the edges of high contrast visible. Fine detail with low contrast is usually lost to the background. This filter can be used to pull out just the elements having high contrast to the image background.

The Sharpening filters accentuate all edges within an image by significantly enhancing all image intensity transitions. This enhancement is accomplished using a technique called "unsharp masking," which essentially sharpens an image by subtracting its low-pass results from the original image. Sharpening is used to bring out fine detail in a image, or to re-focus an image that has been blurred.

The Laplacian filters are edge filters that accentuate intensity changes in an image by modifying a pixel's value to exaggerate its intensity difference from its neighbors. The results are very similar to those of a Hi-Pass filter. It produces an image with harsh intensity transitions, and results in an image with only the edges of high contrast visible.

3.2.4.2.2 Nonconvolution Filters

Nonconvolution filters also work with pixel neighborhoods; however, unlike convolution filters, they do not multiply the neighborhood values by a kernel of filtering coefficients. Instead, a nonconvolution filter works only with the data in the neighborhood itself, and uses either a statistical method or a mathematical formula to modify the pixel upon which it is focused.

The Median filter smooths an image by modifying pixels that vary significantly from their surroundings. This smoothing is accomplished by replacing the center pixel in a neighborhood with the median value of the neighborhood. Although median filter softens an image, it generally preserves its edges. This filter is particularly effective at removing random, high-impulse noise from an image, e.g., spots or points that vary significantly from the background.

The Erosion filters are morphological filters that change the shape of objects in an image by reducing bright objects boundaries and enlarging the boundaries of dark ones. It is often used to reduce, or eliminate, small bright objects.

The Dilation filters are morphological filters that change the shape of objects in an image by enlarging the boundaries of bright objects, and reducing the boundaries of dark ones. The dilation filter can be used to increase the size of small bright objects.

The Opening filters are morphological filters that perform an erosion, then a dilation. In images containing bright objects on a dark background, the opening filter smoothes object contours, breaks narrow connections, eliminates minor protrusions, and removes small dark spots. In images with dark objects on a bright background, the opening filter fills narrow gaps between objects.

The Closing filters perform a dilation followed by an erosion. In images containing dark objects on a bright background, the opening filter smooths object contours, break narrow connections, eliminates minor protrusions, and removes small bright spots. In images with bright objects on a dark background, the closing filter fills narrow gaps between objects.

Robert's filters extract and enhance fine edges in an image by expressing the differences between neighboring pixels as an intensity value. In neighborhoods where there is no difference among values in the neighborhood, the pixel's intensity is set to 0 (black); where there is the greatest possible difference, the pixel is set to 255 (white). Intermediate levels of gray reflect varying amounts of difference. The result is an image in which edges and contours are highlighted against a dark background. Robert's filters enhance all edges within an image, even those introduced by noise.

The Sobel filters extract and enhance edges and contours in an image by expressing intensity differences (gradients) between neighboring pixels as an intensity value. To do so, it combines the difference between the top and bottom rows in a neighborhood with the difference between the left and right columns. The neighbor-intensity is set to 0 (black); where there is the greatest possible difference, the pixel is set to 255 (white). The results are similar to the Roberts filter, highlighted edges against a dark background, but the Sobel filter is less sensitive to image noise.

Therefore the Sobel filter gives an image with smoother, more pronounced outlines of only the principal edges.

3.2.4.3 Frequency Filtering

Image interference (noise) that presents itself as a regular pattern across an image can be especially difficult to remove using a spatial approach. The best way to eliminate such periodic or coherent noise is by converting the image to a set of frequencies, and editing out the frequencies causing the problem.

One of the methods that convert an image to its frequency domain is called a Fourier Transform. The image's frequency domain can be expressed as a symmetrically centered cloud of points, where brightness represents amplitude of the wave form, and the position represents the frequency of the wave form.

In a normal image, the spectrum will appear as a roughly circular cloud that is brighter and denser near its center. Images containing a regular pattern will reflect pattern-like effects in their spectra. It is this characteristic that can be manipulated to remove coherent noise. Coherent noise usually manifests itself as bright points outside the central cloud. Removing these points eliminates the frequency causing the noise.

3.2.5 Image Segmentation

Images normally contain representations of multiple objects. For example, an image of corn roots will show roots and soil. To achieve the goal of an image-processing application, it is necessary to divide the image into regions, each of which represents one of the physical objects that have been imaged. The process of dividing the image up is known as segmentation. We could make an image in which all roots pixels are white and the soil black. We could then determine the number of roots, their length, or their average diameter.

Some of the basic approaches to segmentation are based on finding regions in the image in which the pixels are in some sense similar to one another. Pixels are regarded as similar if they have a similar color or brightness or because they form part of a consistent, repeating pattern or texture. Other techniques are based on trying to identify image features that are consistent with the boundaries between regions or objects. A complete boundary, when found, differentiates one region from others.

3.2.5.1 Edge Detection

Edge detection is one of the commonest approaches to solving the segmentation problem. It is concerned with detecting the boundaries that separate distinct areas. Edge detection relies on the observation that discontinuities in brightness or color are often associated with physical boundaries between objects. Discontinuities are also associated with boundaries between different parts of the same object. Consequently, dividing an image on the basis of these discontinuities should result in segments that correlate strongly with complete objects, or with important parts of objects. It is important that the resulting segments are related to regions and objects in the original scene so that we can extract information based on them. There are many forms of edge detector. Among them the Canny edge detector has been claimed as optimal in terms of its edge location accuracy and its ability to suppress noise.

3.2.5.2 Segmentation by Region Growing

Region-finding techniques are based on the absence of discontinuities within the regions themselves. The region-based approaches try to form clusters of similar pixels. Initially, clusters can be formed by grouping adjacent pixels with the same value. The clusters are enlarged by merging neighboring clusters. The merging stops when the neighboring clusters fail to meet some measure of similarity.

3.2.5.3 Classification

Classification is another approach to segmentation. Classification techniques use statistical measures of the image to partition pixels into one of a small number of classes. Each class is associated with some particular kind of property of the scene that was imaged.

3.2.6 Image Analysis

The goal of image processing applications is some kind of analysis of the image data. The analyses include identification of one or more objects and their measurement. Some of the most important identifiable components of image extraction techniques are based on shape. Shape offers the most direct correspondence between an image and the objects of interest. Template matching can be applied under controlled conditions to locate features. Template matching is based on correlation. The template must contain an image of the feature being searched for. Instead of tying to match image features with templates on a pixel for pixel basis, it is possible to match templates based on parameters derived from the segments themselves. The derived parameters, such as perimeter length or area, can be used to discriminate between known objects, if the values are significantly different.

Image processing applications also let user perform spatial measurements upon an image. The measurements are length, area angle, perimeter, diameter, density, etc. Measurement operations are performed in terms of screen pixel positions, e.g., the area of an object is the total number of pixels within the outline, the perimeter of an object is determined by counting the total number of pixels on its boundary, and so forth.

The hardware of our image analysis system consisted of an IBM 486DX/33MHz personal computer equipped with a video frame grabber-digitizer board (DataTranslation 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 a thinning operation.

All digital image processing except thinning and counting of captured root images was accomplished in the Windows environment using Image-Pro Plus as developed by Media Cybernetics (Silver Spring, MD., USA). This software is tool-bar and menu-driven which allows easy to use. It also have many standard image processing techniques and analysis tools, such as filtering, histogram equalization, FFT (fast Fourier transform), and counting and measuring parameters of the object.

3.3 Measuring Root Parameters

Root number and average diameter can be easily measured by using image processing software (Image-Pro Plus). The application has several analysis functions including number and average diameter measurement. However, this software cannot do the thinning operation and then count the number of pixels in the structure. A program that can thin the root to one pixel thick and then count the number of pixels in the structure should be developed.

3.3.1 Preprocessing

Root images may need enhancement before root parameters are measured. The enhancement can be a simple contrast adjustment or a more complicated manipulation. Usually an image contains unnecessary signals (noise), and the noise should be removed before any further image analysis.

When photographed against a dark soil background, roots generally appear brighter. By examining the frequency distribution of bright intensity value, the histogram, of a digitized root image, the root point values can be distinguished from the background point values. The image processing system can separate the image into its root and background components when an appropriate threshold value is selected. Points whose bright value is greater than this threshold are considered part of the roots and are set to white, pixel value 255. Points whose bright value is less than the threshold represent soil and are set to black, pixel value 0. The thresholding process is used to provide high contrast (black and white) between roots and soil.

3.3.2 Skeletonization

The thresholded binary images which have 2 gray-scales (roots white and background black) are skeletonized. Skeletonization is a binary image transformation that may be implemented using formation cellular logic (morphologic) operation, and results in a one-pixel-thick line with the same topology as the original object.

Skeletonization of an object is often referred to as the Medial Axis Transform. 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. Lebowitz (1988) and Smit et al. (1994) showed the usefulness of the skeletonizing method to measure root lengths.

The MAT may be explained by reference to a grass fire (Blum, 1967 -- Quoted by Myler and Weeks, 1993). When fire is started on all the contour of the grass field simultaneously, the fire proceeds to burn toward the center of the field until all of the grass is consumed. The fire lines would meet and the fire would be quenched. The points that the fire lines crossed and are then extinguished are known as the medial axis of the object.

Morphological skeletonization is defined as the union of the set of pixels computed from the difference between the nth eroded image and the opening of the nth eroded image (Myler and Weeks, 1993). The skeleton image is then given by the union of all differences over all erosions. The total number of erosions, N, required by the skeleton algorithm is the number of erosions of the original image by the structuring function that yields the null image.

3.3.3 Image Analysis System Calibration

Image analysis system calibration is necessary to express root length by terms of spatial unit (cm) rather than by number of pixels. To calibrate the image analysis system, known lengths of thread or wires are usually used (Lebowitz, 1988; Cunningham et al., 1989; Shuman et al., 1993).

In the study of methods for measuring the length and diameter of small root samples, Lebowitz (1988) expressed the root length as a function of D, the distance between horizontally or vertically adjacent points in the root image lattice. The digital image analyzer was calibrated by repeated scans using a set of standards of known length. The value D was computed by the regression equation.

$$L_i = A + B \times ((H + V) + (2)^{0.5} \times X) \qquad (2)$$

where L_i is the length of the ith standard, 'A' is the location parameter, which should be zero but would actually be nonzero due to sampling error; 'B', the slope of the regression, B is the least squares estimate of D; H, V, and X are the number of horizontally, vertically, and diagonally adjacent pairs of points in the medial axis of the roots. He distinguished the number of diagonally adjacent pairs of points because the distance between them is the square root of 2 times greater than the distance between vertically or horizontally adjacent pairs (Pythagorean theorem).

Smit et al. (1994) criticized Lebowitz's approach because it does not, when averaged over all possible directions, give a correct weighting factor on average for pairs of pixels. Under the condition of random orientation of roots, it is not correct to take the average of $\sqrt{2}$ and 1 $((\sqrt{2}+1)/2 = 1.21)$ as the correction factor. He proposed a theoretical value for the correction factor c in the interval 0 to π/4 based on the following formula.

$$C = \left[\int_0^{\frac{\pi}{4}} \left(\frac{d\alpha}{\cos\alpha}\right)\right] \Big/ \left(\pi/4\right) = 1.12 \tag{3}$$

where α = the angle of line segments to a horizontal line. By his correction factor the total root length per image could be calculated by

$$L = (N \times C) / r \tag{4}$$

where L = the length of roots in cm, N = number of skeleton pixels, and r = the resolution in pixels per cm.

To evaluate image processing system characteristics, a preliminary measurements was made with known length. A 5 cm horizontal line drawn on a paper as a standard; below the standard line, several lines totalling 65 cm were drawn in random directions. To calculate the system resolution in pixels per cm, the 5 cm horizontal line was measured by the Image-Pro Plus length measurement function. After that, the randomly drawn lines were thinned and the number of pixels counted. With the value of the resolution in pixels per cm, the number of pixels of the one-pixel-thick structures of the random lines were converted to a spatial unit (cm). The estimated length was about 4 % longer than the actual length, which was measured manually by ruler. If Smit's correction factor, c = 1.12, is adapted, the error could increase as much as 16 %. To investigate the necessity of the correction factor, three horizontal and three diagonal 5 cm lines were measured in pixels. The average number of pixels in both horizontal and diagonal 5 cm lines was 382. Theoretically, the number of pixels in a diagonal line should be $\sqrt{2}$ times less than the number of pixels in the same length of horizontal lines. This ratio is called as aspect ratio (Media Cybernetics). Therefore, the resolution in pixels-per-cm value of the image processing system is obtained empirically.

In this study, a standard ring was used to calculate the resolution 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:

$$RL = r \times N \tag{5}$$

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

3.3.4 Root Image Skeletonizing Program

The root image skeletonizing program is made with MATLAB. The skeletonization program had to be developed because the main image processing software, ImagePro Plus, does not have the function of skeletonizing and then counting pixels.

3.3.4.1 MATLAB

The MATLAB (matrix laboratory) is a software for numeric computation and visualization. It was originally written for easy matrix computation, but has evolved over a period of years with input from many users. Now it integrates numerical analysis, matrix computation, signal processing, and graphics in an easy-to-use environment. MATLAB also features a family of application-specific solutions called toolboxes. Toolboxes are comprehensive collections of MATLAB functions (M-files) that extend the MATLAB environment in order to solve particular classes of problems. Areas in which toolboxes are available include signal processing, control systems design, dynamic systems simulation, systems identification, neural networks, image processing, and others. MATLAB's easy extensibility allows users to develop new applications easily. Among the tooboxes, the Image Processing Toolbox is very useful for developing a skeletonizing program. The Image Processing Toolbox is a collection of functions built on MATLAB's numeric computing environment. The toolbox functions support a wide range of image processing operations, from image display to filtering, analysis, and image transforms. Most of the functions in the Image Processing

Toolbox are MATLAB M-files, a series of MATLAB statements that implement specialized image processing algorithms. The way the toolbox function works can be changed by copying and renaming the M-file, then modifying the copy. Also, the toolbox can be extended by adding new M-files.

3.3.4.2 Image Analyzer

The skeletonizing program called Image Analyzer is developed by using several M-files of the Image Processing Toolbox. The Image Analyzer has a graphical user interface (GUI) made up of graphical objects, such as menus, buttons, and lists. This GUI is implemented to provide easy use and to reduce image handling time. The Image Analyzer lets users select the opening image, saving image, skeletonizing, thinning, finding perimeter, and counting pixel number options. The *opening image option* allows the user to retrieve an image to the application. The *saving image option* saves the processed image to storage media. The opening and saving options support a GIF-type image. *Skeletonizing* and *thinning options* invoke skeletonizing and thinning functions in the Image Processing Toolbox, respectively. Both skeletonizing and thinning functions skeletonize the object to its basic structure. However, the result of preliminary tests shows a tendency for the skeletonizing function to overestimate the actual sewing thread length. But the thinning function allows the thread length to be estimated close to the actual thread length. Therefore, the thinning function was used to skeletonize the root image in this research.

When the thinning option is selected, a binary image morphological operation is performed by the bwmorph function. The bwmorph function filters a binary image using the filter h=2.^[0 3 6; 1 4 7; 2 5 8]. This filter has 512 possible results, one for each possible pixel configuration in a 3-by-3 neighborhood. The filtering process reshapes a 3-by-3 binary neighborhood into a 9-bit integer. Bwmorph uses the result of the filtering as an index into a 512-location lookup table that contains the desired output for each possible pixel configuration. Typically, a lookup table contains 1s for "hit" pixel configurations and 0s for the remaining "miss" configurations. The operation that bwmorph implements is called a *hit-or-miss transform*. The resulting value appears in the center of the 3-by3 neighborhood. By selecting the appropriate lookup table, the bwmorph applies a specific morphological operation to the binary image.

The *perimeter option* returns the perimeter of objects in the binary image. It is used to find the relationship between the number of pixels and the known length of the perimeter of the standard disk. With this relationship and the number of pixels of the thinned root basic structure, root length is calculated. To find the number of pixels of the thinned root basic structure, *the count option* is implemented in Image Analyzer.

3.3.5 Length Measuring System Evaluation

The length measurement system by image processing method is checked with a known lengths of white sewing thread. Several thread length samples are used to find the relationship between actual thread length and estimated length. A total of 11 sample lengths are measured for each 10 cm increment from 10cm to 100cm and 140 cm. For each sample, the thread is cut into several pieces and dispersed randomly over the black paper. Four images are taken for each sample by turning the black paper on which thread is laid. The thread images are enhanced by adjusting brightness, contrast, and gamma value. Enhanced images are thresholded to distinguish thread from background. The thresholded binary images are thinned to their basic structure. Finally, the number of pixels in the structure are counted and the total used to calculate the length of thread.

3.3.6 Measuring Root Length Density *in situ*

The main problems in rapid estimation of root length are the large labor involvement in removing the roots from the soil and in measuring of lengths of washed roots. The time required for measuring the length of washed roots are continuously being decreased with the adoption of new techniques, such as Newman's line-intersect method and the image processing method. However, the washing procedure in plant root research is still the most time-consuming part. Time for root research can be dramatically reduced, if the root length density can be estimated with reasonable accuracy without washing the roots. Two different approaches were tried to measure root length density without washing roots from soil. One approach used root number, the other used root length on the soil profile.

3.3.6.1 Using Root Number

Root length density can be calculated by counting the number of roots intersecting a soil plane of known area. Melhuish and Lang (1968) first proposed this method. They used the number of intersections of roots within soil plane surfaces to calculate root length density based upon geometrical probability. Melhuish impregnated soil cores with a polyester resin and then ground the plane surfaces with the cutting saw to analysis the roots. Bragg et al.(1983) compared four methods of measuring root distribution including the core-break root count method in which root density is estimated from the number of roots on the horizontal faces of core segments. Root densities estimated by core-break count were highly correlated with lengths of roots washed from soil. They confirmed that relative root distribution at a particular growth stage can be assessed from counts in the field of the number of roots crossing horizontal faces in core segments. The method in principle is very attractive but the impregnation of the soil and the grinding procedure are very tedious and time-consuming. This drawback prevent Melhuish's method from being used when a large number of samples are to be measured. Moreover, root systems in soil are not completely random, contrary to the assumption of the Melhuish and Lang method. Using Melhuish and Lang's equation with a fixed coefficient to calculate root length density in a non-random rooting condition might produce error. In this rooting condition, when Meyer and Barrs (1985) found a relationship between root number and root length density they got larger coefficients than those in Melhuish and Lang's theoretical equation. Another source of error is the difference in the number of roots that are counted by different observers.

3.3.6.2 Using Rooting Intensity

Root length density also can be estimated directly, instead of by counting root numbers. One method quantifies the root length density by measuring the length of roots that lie on the cut soil surface. The root length visible per cm^2 of soil surface is termed rooting intensity.

Bohm (1976) estimated root length density by rooting intensity. He calculated the rooting intensity by counting a 5 mm-long root unit as one root. Root length was calculated in a distinct soil volume based on the uniform distribution of roots in the soil. If a 5 mm thick block of soil is removed from a 5 × 5 cm square soil profile, and if one estimated root unit is equivalent to 5 mm in root length, a total count of 2 root units in the soil profile corresponds to a root length of 800 m/m^3. Bohm(1976) compared this estimated root length with the root length measured with a root-counting machine after the root was washed. His estimated root length was about half that measured after washing. The possible reason for this underestimation is that the fine roots cannot be distinguished at the soil profile because wet fine roots often adhere closely to the larger roots during the counting procedure. Also, some fine roots can be lost during the exposing procedure.

This approach was used mainly with the minirhizotron method. Because of the root concentration effects at the soil-grass interfaces, usually root length density (cm/cm^3) cannot be calculated accurately from the rooting intensity in a rhizotron or a minirhizotron method. However, if root concentrations at the soil-grass interface are slight, such a calculation can be made (Taylor et al., 1970; Klepper et al., 1973). These researchers could calculate root length density from the rooting intensity by knowing the whole soil volume of the rhizotron compartment and assuming the thickness of soil layer that can be seen behind the observation windows. They assumed that all roots within a specific distance of the wall of the tube are seen and that no roots can be seen beyond that distance. The direct estimate of root length density is acquired by dividing a volume which is calculated from the viewing distance and the observed area of tube, into the root length observed at the interface. Sanders and Brown (1978) also used this method in their research. They compared the root length density estimated from a duodenoscope and from the core sample. Root development patterns around a transparent Plexiglas tube imbedded within the root zone were photographed using a fiber optic duodenoscope. Root lengths for soybeans were calculated from the number of intersections between the root image projected onto a viewing screen and a grid inscribed on screen. Root length was converted to the roots length density for the volume represented by the photograph. The volume represented by the photograph was calculated by multiplying the photograph area and the assumed depth of view from the photograph, 0.3cm. Vos and Groenwold (1983) found experimentally the relationship between the amount of roots, viewed by endoscope in the minirhizotron (cm/cm^2), and the root length density (cm/cm^3) in adjacent bulk soil, measured by core sampling. For wheat plants the root length (cm/cm^2, endoscope) = 0.29 times the root length density (cm/cm^3, soil cores). Using this approach with the minirhizotron in root research produces an error created by the properties of the soil-tube interface. Roots can elongate at a slower rate in this zone because of the compaction of the soil around the tube, or roots can elongate faster in this zone because of the void spaces along the tube. The minirhizotron system frequently underestimates rooting at the near-surface (upper 0.2 to 0.4 m). There is no explanation for this phenomenon. Also, the accuracy of this system is low where root density is low (Taylor et al., 1990).

3.3.6.3 Choice of the root quantifying method without washing root

Although there is a certain relationship between root number and root length density, quantifying root length density with the root number has a limitation. To apply this method, the root system has to be distributed randomly and uniformly. In fact, the root system has a preferential growing direction. Because this method is based on the assumption of random and uniform root distribution, in many cases this method produces wrong results. Also, the number of roots may be counted differently by different observers. To estimate more accurately the root length density, a more robust method is needed. The main source of error in estimating root length density using rooting intensity is the different growing conditions at the soil-observation tube interface. If the error from the soil-glass interface is removed, estimating root length density by rooting intensity could be a good candidate for estimating root length density without washing the root. The error can be eliminated by measuring rooting intensity at the non-disturbed soil surface. By exposing roots in their natural growing condition, rooting intensity is measured without interaction between roots and soil-observation tube interface. Hence, in this research, rooting intensity is used to estimate root length density without washing the root.

3.4 Staining

In order to improve the contrast with the background, the roots are stained. Smit et al. (1994) submerged roots in a solution of 50 mg L^{-1} methylene blue for 5 minutes; staining made the roots show a darker gray against a bright background.

Commins et al. (1991) evaluated several staining techniques to enhance the contrast between roots and the resin-impregnated soil block. Their staining materials were Carboxyfluoroescien diacetate, Calcofluor MR2, Tetrazolium salt, Cellufluor, Bisbenzimide, Tetrazolium salt, Aniline blue, Toluidine blue, Safranine orange, and PAS reaction. They also evaluated the usability of Autofluorescence with a BG 38 filter. They concluded that the only effective contrast enhancement method was the manipulation of autofluorescence with the filter BG 38. The method used 360 nm UV light (100 W longwave ultraviolet floodlight lamp, Black Ray Ultraviolet Floodlight Lamp, Model B-100A; Gelman Science, Sydney, Australia) for autofluorescence to cause natural root fluorescence, because in roots of many species blue fluorescence occurres at wavelengths of 310 - 360 nm (Holland and Fulcher, 1971, quoted by Commins et al. 1991). The BG 38 filter was attached to the video camera lens to increase enhancement; also, a 3 mm sheet of clear polycarbonate was used to eliminate reflected UV light.

Ruark and Bockheim (1988) used a 1% solution of Rhodamine WT dye (Crompton and Knowles Crop. of Skokie, Illinois) as a staining method for their study. They sprayed a 1% solution of Rhodamine WT dye, an anionic, maroon-colored dye commonly used in water tracing (WT) studies. The dye darkly stained organic layers and roots against a more lightly stained soil matrix because it was preferentially absorbed by roots and organic matter, while being essentially repelled by soil particles. A Kodak Wratten 15, dark yellow filter was attached to a 35 mm camera loaded with color infrared slide film (ASA 100) to filter out light below 520 nm. The processed 35 mm slides made the bright yellow roots contrast well with the dark brown soil matrix.

In our study, Commins et al.'s (1991) autofluorescence method was used because the method is easy and can stain roots instantly. The quick staining prevents roots from drying. The easy of use of this staining method is suitable for video camera-based image acquisition.

3.5 Experiment

We need information about the root system in the bulk soil but must use the root in the surface of a cut soil sample. Therefore, the parameter measured at the cut soil surface should be directly related to the bulk soil root density. An experiment was performed to find the relationship between these two parameters. Figure 3.1 shows the schematic diagram of the root image recording system.

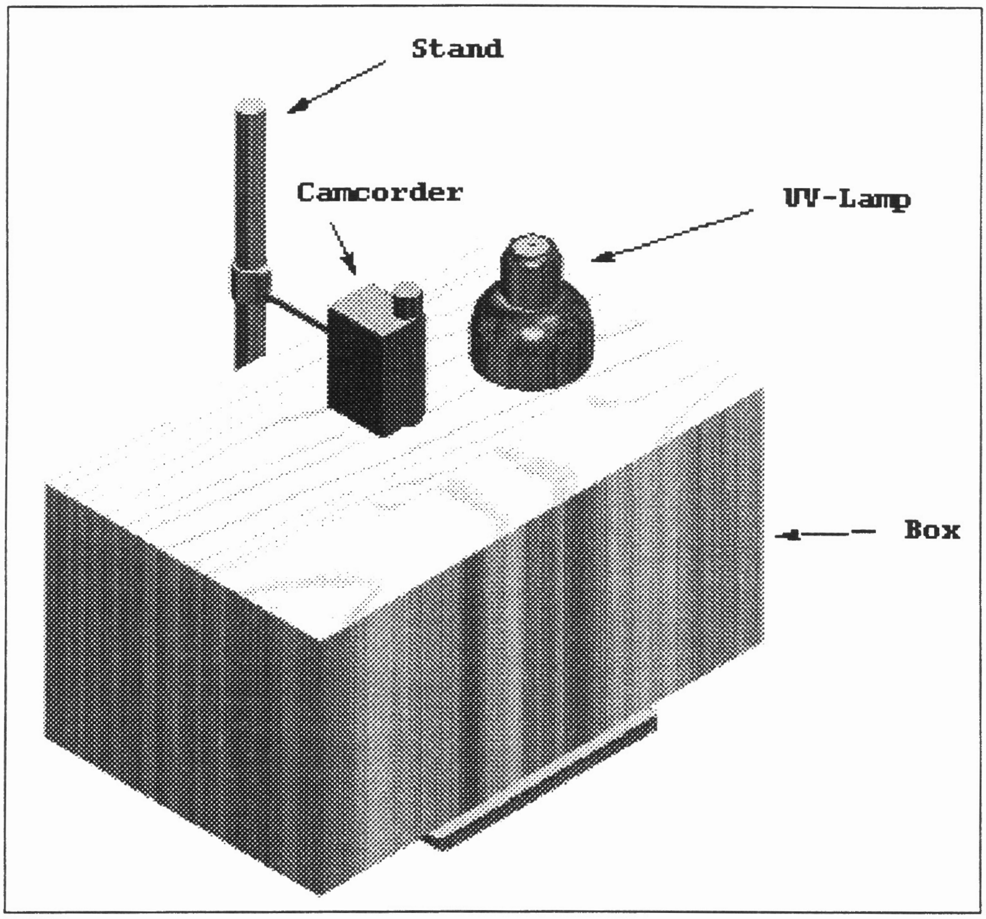

Figure 3.1 Schematic diagram of the root image recording system

3.5.1 Procedure

The root system of a corn plant was used to obtain samples for estimating root length. Root samples were acquired by core sampler. The soil sample was contained inside two pieces of half liners. The soil core was cut along the length by a sharp knife into two pieces. Roots were exposed at the cut soil surface, and root images recorded by camcorder. The root images were downloaded to computer, and the root length on the cut soil surface was measured by image processing. After being photographed, the root-soil core sample was put into a plastic bottle. The roots were washed from the soil, and images were taken by CCD camera in the laboratory. The length of washed roots was measured by image processing and paired with the root length on the cut soil surface. Finally, the relationship between these two lengths was determined and used to estimate root length density. Figure 3.2 explains the difference between this method and other roots observation methods. Each method has its own advantages and disadvantages. Thus, this method combined the most important components effectively.

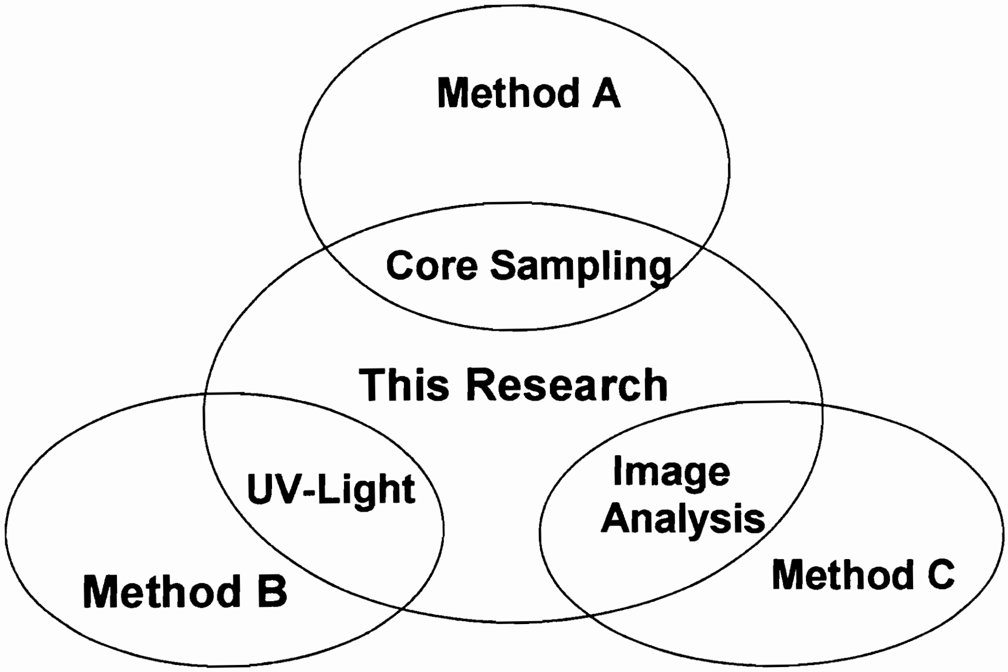

Figure 3.2 The difference between other root observation methods and this method

3.5.2 Root Sampling

Roots can be exposed by several sampling methods, such as excavation, monolith, auger, profile wall, and core-sampling. Among them, the core-sampling method is the most important in ecological root research because the sampling can be done quickly without significantly damaging the plants to be investigated. To take the root samples, a handheld core sampler was used.

3.5.3 Root Exposing

An aluminum liner which contains soil and root was carefully separated from the core sampler. The soil core was divided into two pieces along the length by a sharp knife. A plastic cap was placed on the ends of cut soil core to prevent soil crumbling at the edges. A soil layer about 2 mm thick was removed and the roots exposed with a paintbrush and a scraping tool, a very thin driver. Water was sprayed to enhance the contrast between roots and soil. The root images were taken immediately after exposing to prevent drying.

3.5.4 Root Image Recording and Downloading

The exposed root sample was placed on the black plastic plate, which was then placed in a cardboard box which provided dark lighting conditions and supported the UV lamp. Root autofluorescence was caused by illuminating the UV-lamp. The root image was recorded for about 10 sec. Then the sample was turned 90° and the image recorded again. In this way, four images in four different positions were recorded for each piece of the sample. The remaining piece of the sample was placed under the camera and the images recorded. Then the sample was placed in a bottle containing water.

The recorded images were downloaded to computer on the same day. To download images, the video-out port of the camcorder was connected to the video-in port of the frame grabber. Root images were acquired by the Image Average option of Image-Pro Plus software. Image averaging was done to reduce temporal or random noise in the image.

3.5.5 Preserving and Washing Root

Soil samples for which images were recorded in the field were suspended in water and stored in a cooler before being moved to the laboratory. Roots were washed out from the soil samples immediately after the samples were moved into the laboratory. The soil samples were placed in pails with water. The soil-root-water mixture was stirred until it was a homogeneous suspension. After a few seconds to allow the heavy soil particles to settle, the suspension -- without the settled soil particles -- was poured onto the sieve. Roots remaining on the sieve were washed by clean water to remove adhering soil particles. After the roots were separated from the soil, dead roots or other organic plant debris was removed with tweezers. Live roots can be distinguished from dead roots by their color. Live roots are white, yellow, or brown, and dead roots are gray or dark brown. The washed roots were spread on a black plastic plate and put under CCD camera installed in the lab. Root images were acquired by the Acquire option of Image-Pro Plus.

3.5.6 Processing Root Image

Downloaded images from the camcorder were enhanced by adjusting brightness, contrast, and gamma value. A Low Pass filter was applied to eliminate spot noise. Enhanced images were thresholded to separate roots from the soil background. Several threshold values were used in an image for several region which had different brightness values, because thresholding the whole region of an image with one threshold value doesn't separate roots from soil appropriately.

Root images taken in the lab have good contrast, so the images don't need much enhancement. Merely changing the gamma value to 0.3 allows roots to be distinguished from the background. In this case, using one threshold value was enough to differentiate root.

After thresholding, the binary root images were thinned and 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 ratio. Figure 3.3 shows the root image processing steps.

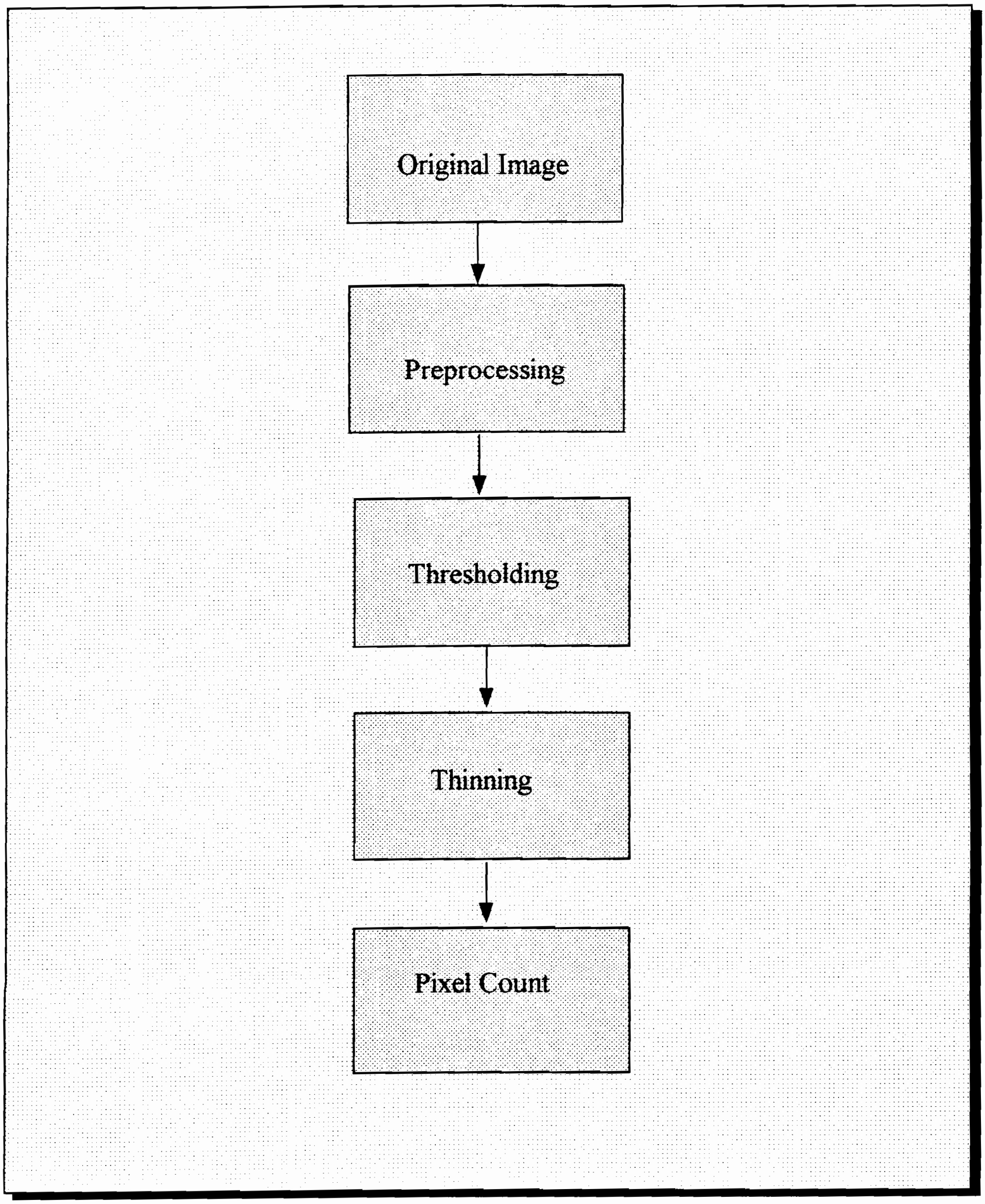

Figure 3.3 Root image processing steps

3.6 Analysis

The root length density measurement method was assessed by examining the correlation coefficient of the linear regression equation acquired from the pairs of the rooting intensity and root length density. Most new root length density methods have been evaluated by this way. Vos and Groenwold (1983) found a simple linear regression equation from the pairs of the means of two endoscopic observations (cm/cm^2) and the mean roots length density (cm/cm^3) of two soil cores taken at either side of the observation tube on the same spots. Bragg et al. (1983) acquired core samples from four blocks, one core per block. The number of roots on the horizontal faces of core segments at 5 cm depth increments were counted and averaged for four cores. The average root intersection numbers were paired with average root length densities measured from washed roots at each depth. A linear regression equation was found from these data to estimate root distribution. Meyer and Barrs (1985) obtained the linear regressions between root intercepts per observation tube and root length density by combining these two values along the depth. They used four replicates core samples for each depth increment.

Root systems have a high degree of variability that is related to inherent properties of the soil-plant system. Roots are not uniformly distributed throughout the soil because of non-uniformly distributed properties of soil and the tendency of the root to follow the path of least resistance. When the rooting intensity is low, the variation is increased. Usually many samples are averaged for root length density by observing the density at a certain number of points. According to Upchurch and Ritchie (1983), the correlation coefficient between minirhizotron observations and root length density determined by soil cores was increased when the average of eight tubes and eight cores was used rather than when only one minirhizotron and one soil core were used (from r value 0.16 (n=796) to 0.81 (n=84)). The increased sample size reduces the variance and produce higher correlation coefficients.

However, the increased sample size requires more time and labor. Certainly, it is against our research goal, which was to find a quick and reliable root length density measurement method. Also, the purpose of this experiment was to evaluate the root length density measurement, not to find the effect of a certain treatment on a plant. Therefore, variability from the non-uniformly distributed properties of soil was not considered in this experiment. For that reason, only one core sample was used to make a pair for determining rooting intensity and root length density. However, eight images per one core sample were averaged to minimize the error from the measuring method itself.

Root length data are usually obtained from different depths. Sanders and Brown (1978) compared the root lengths determined for soybeans by the duodenoscope and by the core method at four depth-intervals. The depth intervals were 0-18, 18-36, 36-54, and 54-72 cm. Root lengths from the duodenoscope were compared with the mean root lengths of two replications of the core at each depth interval. Root samples from different soil depths are acquired in order to determine the root distribution pattern and to find any diversity of RLD values. To increase the range of root length density values, samples were taken from several different positions. Three corn plants were selected. For each corn plant, six core samples were taken. Root samples were taken 10cm, 20cm, and 30cm away from the corn stem. At each distance, samples were taken for the two depth ranges 0~13 cm and 13~26 cm.

IV. Results

4.1 Length Measuring System Evaluation

The objective of the calibration effort was to relate the information of the pixel numbers to the spatial unit, cm. Using a standard ring that has a known circumference, the ratio of pixels/cm was easily calculated for different image acquisition setups. This empirical calibration method eliminated additional effort for considering the image aspect ratio. Figure 4.1 shows the binary image of the standard disk. The inner and outer perimeters of the standard disk are shown in Figure 4.2. The pixel numbers of the perimeter were counted by Image Analyzer and used to divide the known circumference to find the ratio of cm/pixel. One pixel on a root image represents a range of 0.0136 ~ 0.0187 cm depending on camera focal length.

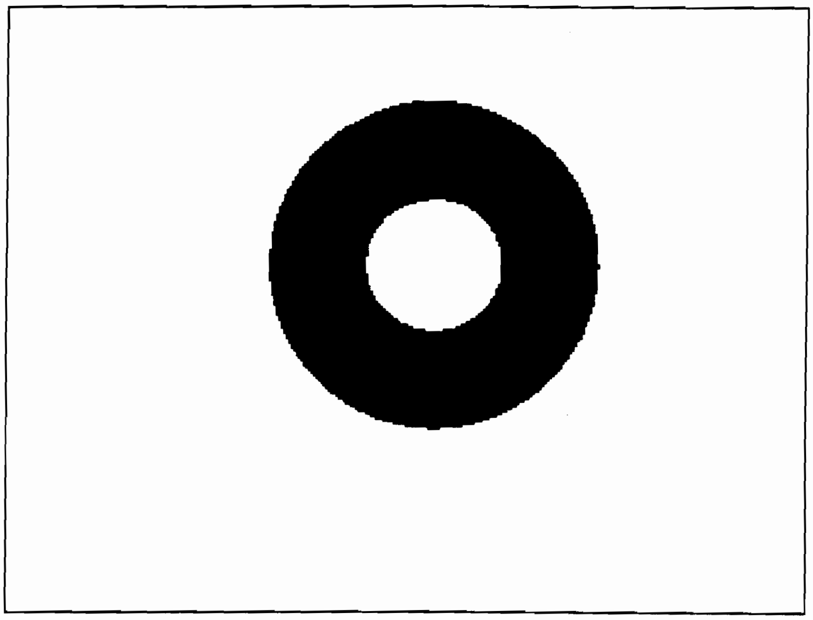

Figure 4.1. The standard ring.

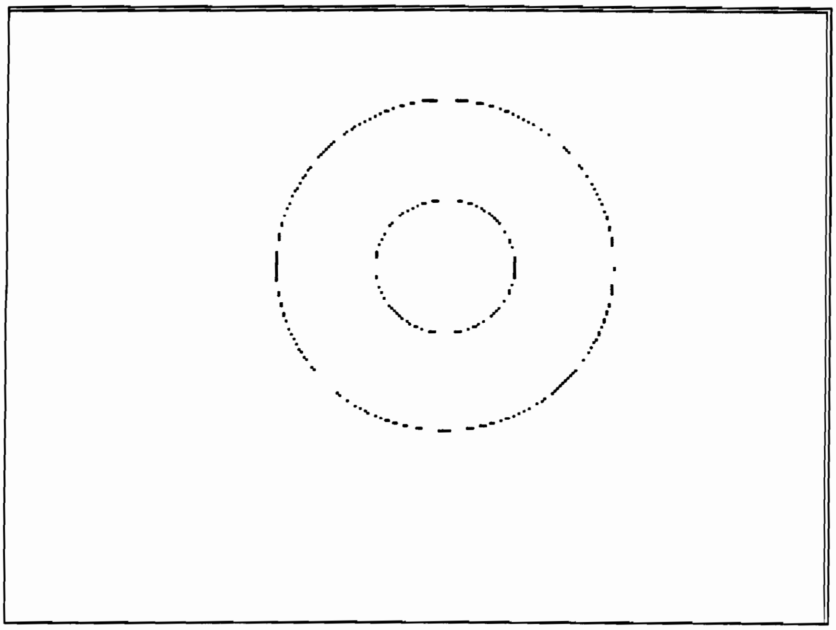

Figure 4.2. Perimeters of the standard ring.

The length measuring systems was evaluated by comparing thread lengths measured by the image analysis system with actual thread lengths. The image of a sewing thread sample and its thinned image are shown in Figures 4.3, and Figure 4.4.

The actual and estimated thread lengths are compared in Table 4.1 and Fig. 4.5. The image analysis method estimated approximately the same lengths for the given samples. Based on these data, regression for the measured thread lengths on the actual lengths was calculated. The regression coefficients are summarized in Table 4.2.

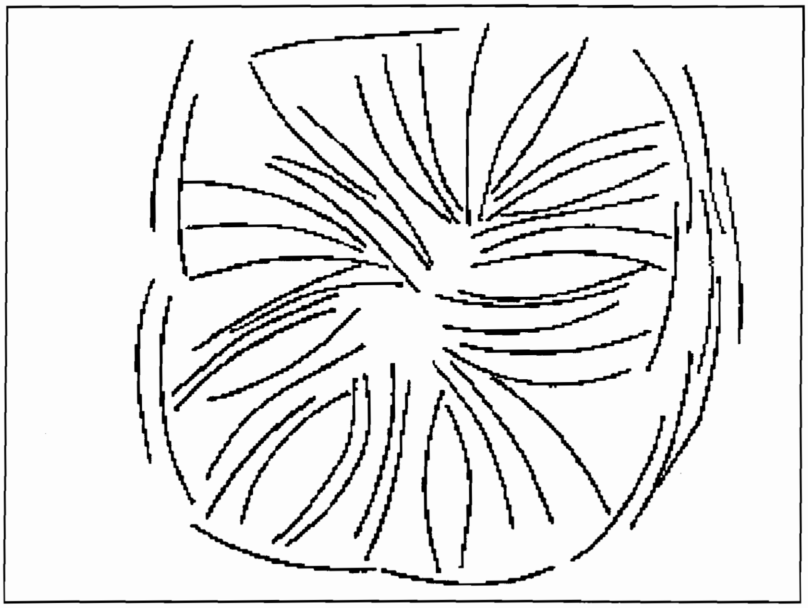

Figure 4.3 Sewing thread sample. (140 cm)

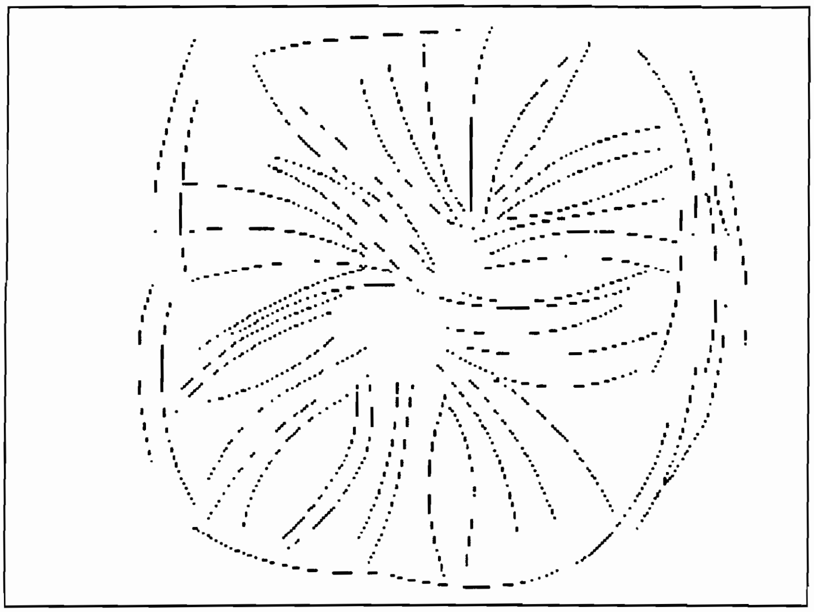

Figure 4.4. Thinned thread sample image (140 cm).

Table 4.1. Comparison of the measured thread lengths with the actual thread lengths.

Sample Number	Actual Length(cm)	Estimated Length(cm)	Error(%)
1	10	10.070	+0.70
2	20	19.755	-1.22
3	30	29.870	-0.43
4	40	39.874	-0.32
5	50	49.807	-0.39
6	60	59.199	-1.33
7	70	69.872	-0.18
8	80	79.632	-0.46
9	90	88.764	-1.37
10	100	99.007	-0.99
11	140	141.672	+1.19

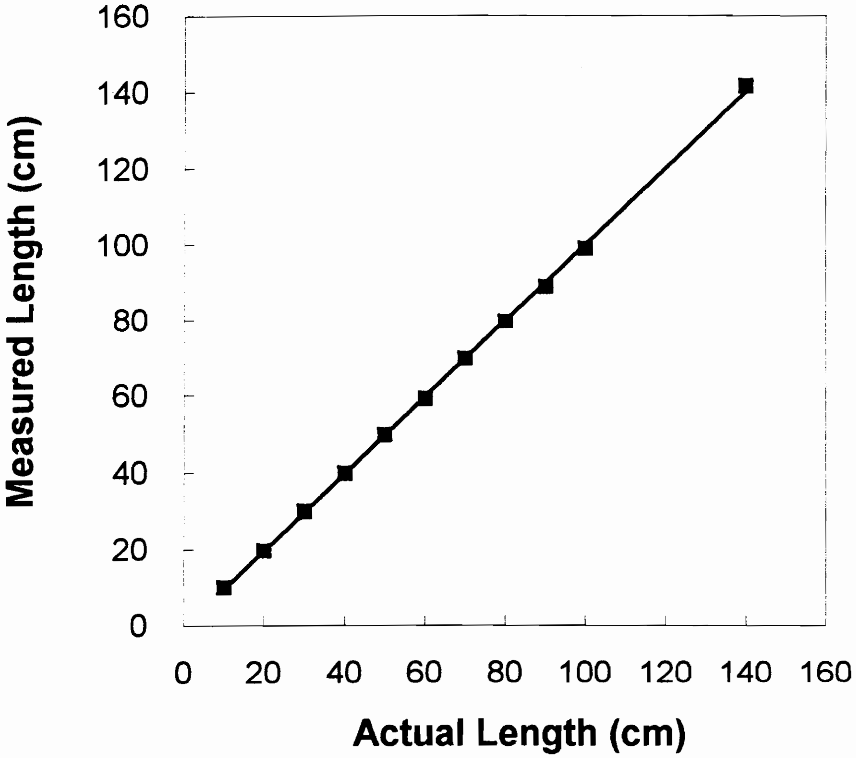

Figure 4.5. A comparison of the measured lengths with actual lengths of sewing thread.

Table 4.2. Relationship between measured thread lengths and actual thread lengths.

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

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 length and the estimated length derived from the image analysis. The image analysis method appears to be reliable. It's more precise than the Line Intersection method. The Line Intersection method used in Reicosky et al.'s (1970) paper estimated thread length with a maximum error of 14.7%. The image analysis method used in this research, which resulted in less than 2% error, is comparable to Lebowitz's (1988) Direct Discrete measurement, which utilized the image thinning algorithm. The calculated maximum error from Lebowitz's paper was 3.2%.

The method examined here is applicable for measuring the length of roots that will be separated from field soil samples. By using this measurement method the time needed for root measurement can be substantially reduced.

4.2 Field Experiment

The analysis of the data set involved calculating correlation coefficients pairing the Root Length Density from washed root samples with the corresponding Rooting Intensity from the planar soil face. The linear relationship between Root Length Density and Rooting Intensity was expected. Results showed the expected linear relationship. Rooting Intensity varied propotionally with Root Length Density. The different number of roots at the cut soil face are shown in Figure 4.6 (Rooting Intensity, 6.43 cm/cm^2) and Figure 4.7 (Rooting Intensity, 1.75 cm/cm^2). Their thinned images are shown in Figures 4.8 and 4.9. Figures 4.10 and 4.11 show the washed root samples corresponding to Figures 4.6 and 4.7, respectively. The thinned images of Figures 4.10 and 4.11 are shown in Figures 4.12 and 4.13.

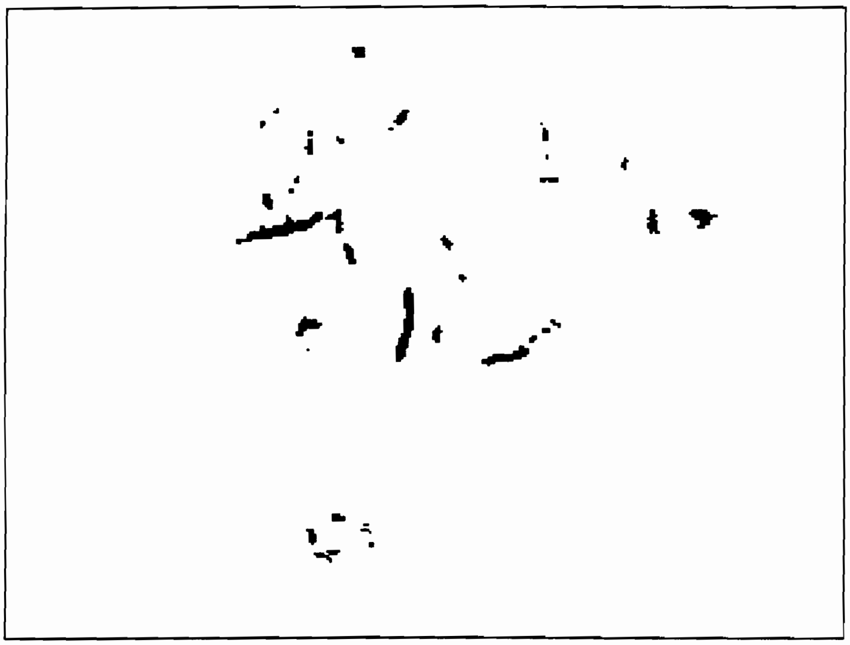

Figure 4.6. The root image at the cut soil surface (Rooting Intensity 6.43 cm/cm^2)

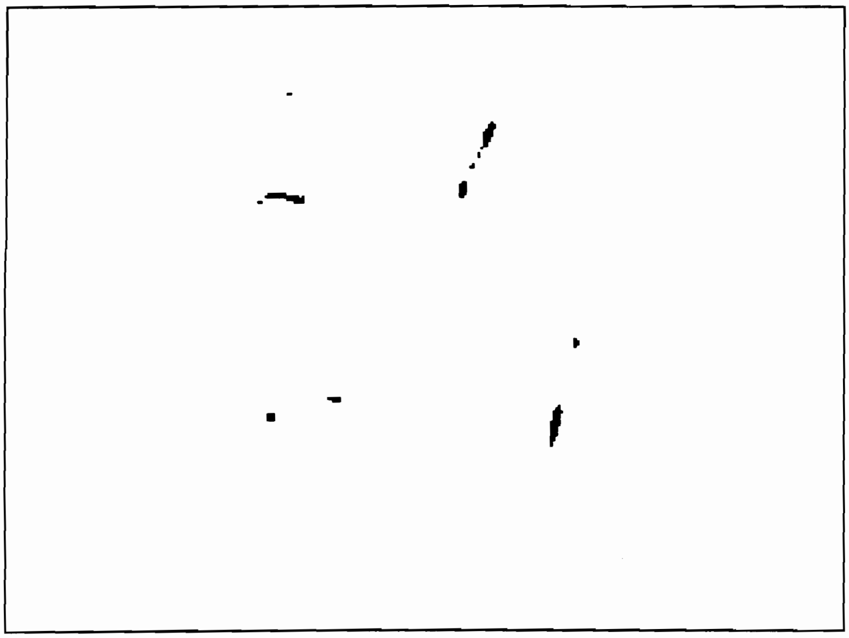

Figure 4.7. The root image at the cut soil surface (Rooting Intensity 1.75 cm/cm^2)

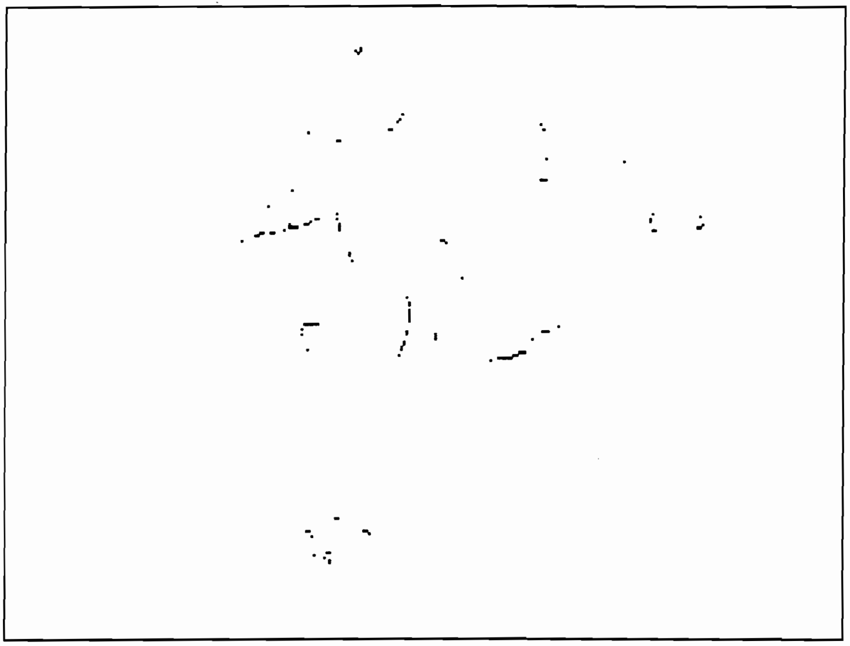

Figure 4.8. The thinned root image, Rooting Intensity 6.43 cm/cm^2 (See Fig. 4.6).

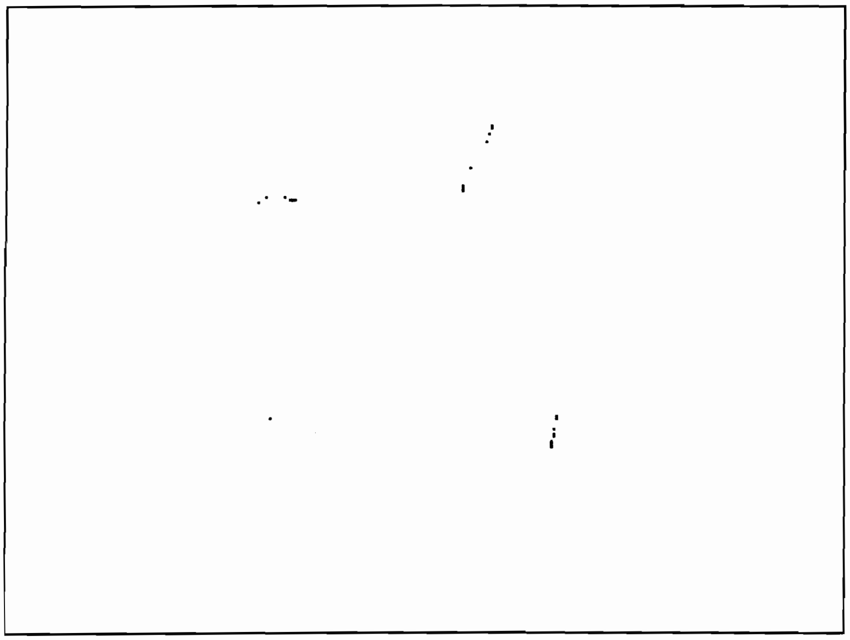

Figure 4.9. The thinned root image, Rooting Intensity 1.75 cm/cm^2 (See Fig. 4.7).

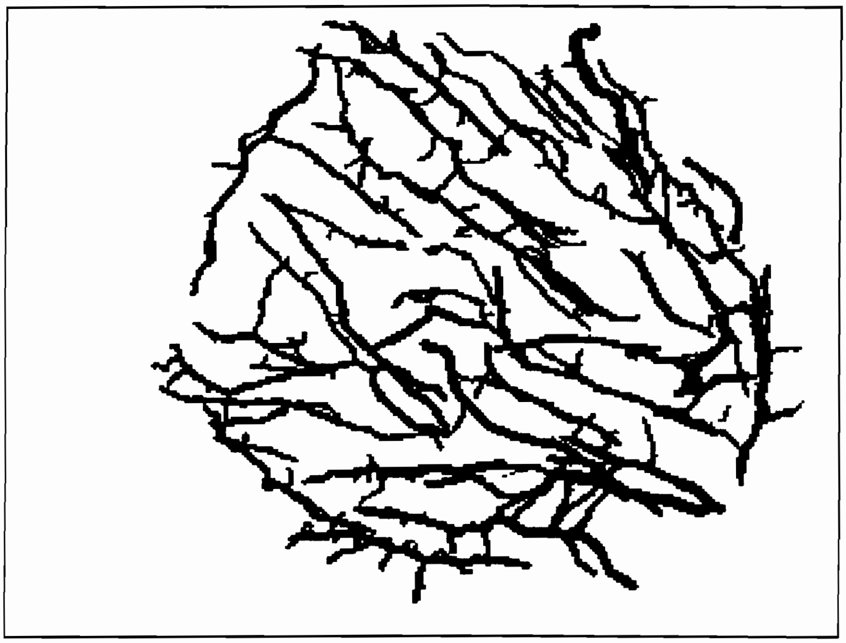

Figure 4.10. The washed root image, Rooting Intensity 6.43 cm/cm^2 (See Fig. 4.6).

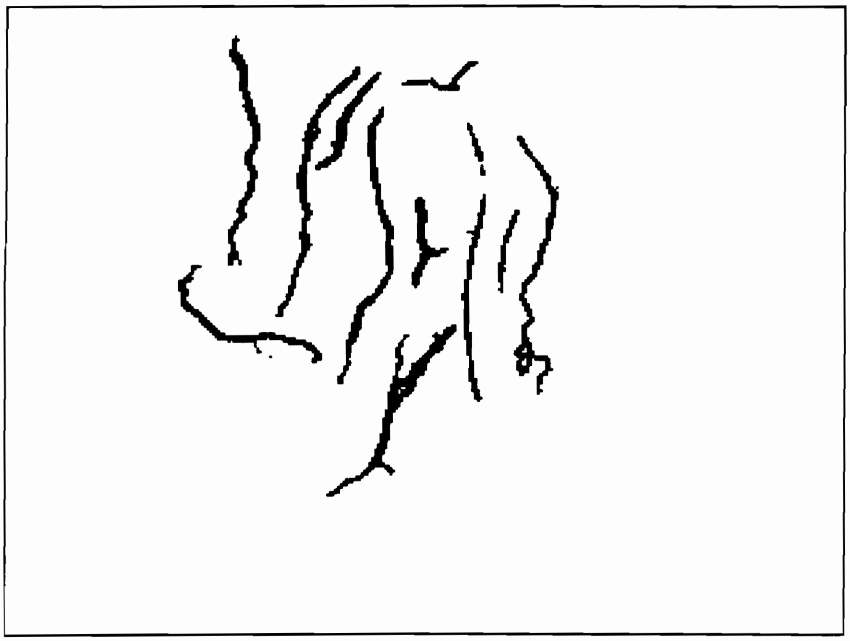

Figure 4.11. The washed root image, Rooting Intensity 1.75 cm/cm^2 (See Fig. 4.7).

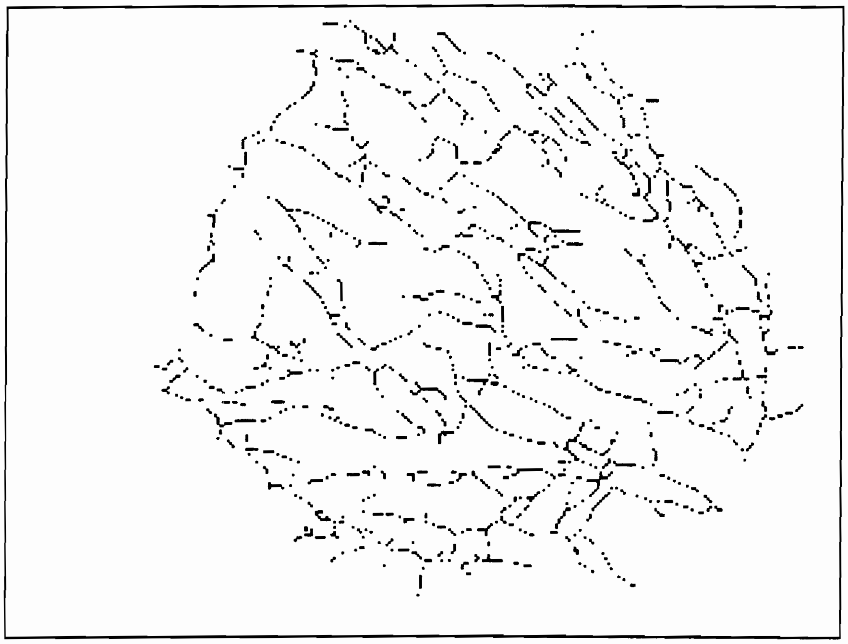

Figure 4.12. The thinned image of the washed root sample (Rooting Intensity 6.43 cm/cm^2) (Fig. 4.10)

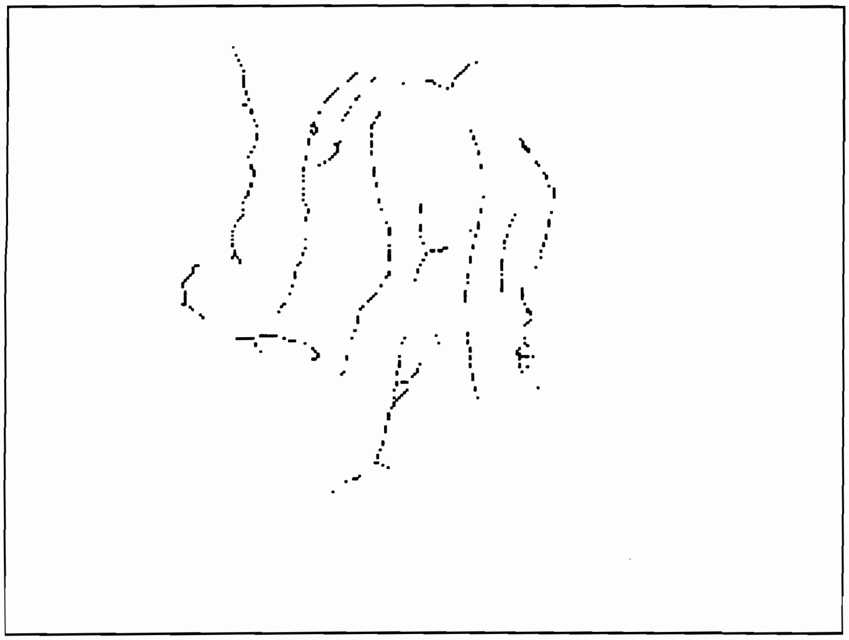

Figure 4.13. The thinned image of the washed root sample (Rooting Intensity 1.75 cm/cm^2) (Fig. 4.11)

Rooting Intensity and the corresponding Root Length Density are summarized in Table 4.3. The linear relationship between Rooting Intensity and the corresponding Root Length Density are shown Figure 4.14.

Table 4.3. Summary of Rooting Intensity (cm/cm^2) and Root Length Density (cm/cm^3) data.

Sample Number	Rooting Intensity(cm/cm^2)	Root Length Density(cm/cm^3)
1	0.254	1.634
2	0.070	0.371
3	0.361	1.978
4	0.026	0.356
5	0.283	1.784
6	0.042	0.433
7	0.218	0.940
8	0.034	0.219
9	0.271	1.782
10	0.052	0.511
11	0.332	1.975
12	0.036	0.317
13	0.074	0.664
14	0.024	0.180
15	0.066	0.546
16	0.051	0.436
17	0.202	1.264
18	0.140	0.842

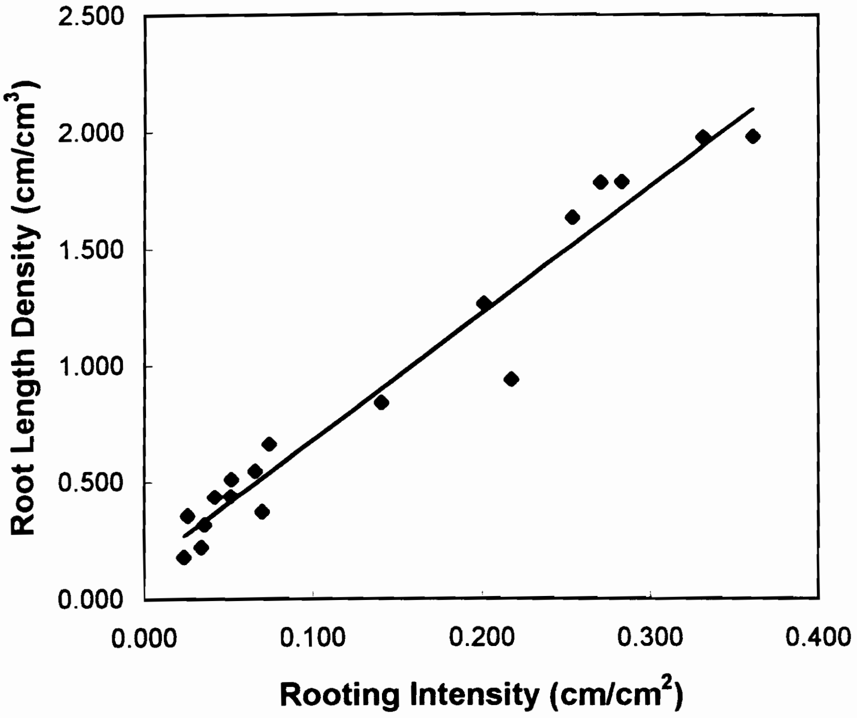

Figure 4.14. Relationship between Rooting Intensity (cm/cm^2) and measured Root Length Density (cm/cm^3).

Table 4.4 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. Table 4.4 shows that Root Intensity is highly correlated with Root Length Density (R^2 is 0.959).

Table 4.4. 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

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 attempted. 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. The removed soil volume was calculated using AutoCad Solid modeling, which can find several properties of a solid model. The Root Length Density then could be estimated by the equation.

Root Length Density (cm/cm^3)

= Root length at soil face (cm) / Removed soil volume (cm^3) (6)

Table 4.5 compares the results of the estimated Root Length Density (cm/cm^3) calculated from the root length at the cut soil face and the measured Root Length Density (cm/cm^3) from washed roots. Figure 4.15 shows the relationship between estimated Root Length Density (cm/cm^3) and measured Root Length Density (cm/cm^3).

Table 4.5. Summary of Measured and Estimated Root Length Density (cm/cm^3) data

Sample Number	Estimated RLD (cm/cm^3)	Measured RLD (cm/cm^3)
1	1.272	1.634
2	0.351	0.371
3	1.808	1.978
4	0.130	0.356
5	1.419	1.784
6	0.209	0.433
7	1.089	0.940
8	0.170	0.219
9	1.357	1.782
10	0.259	0.511
11	1.659	1.975
12	0.178	0.317
13	0.370	0.664
14	0.118	0.180
15	0.329	0.546
16	0.257	0.436
17	1.009	1.264
18	0.703	0.842

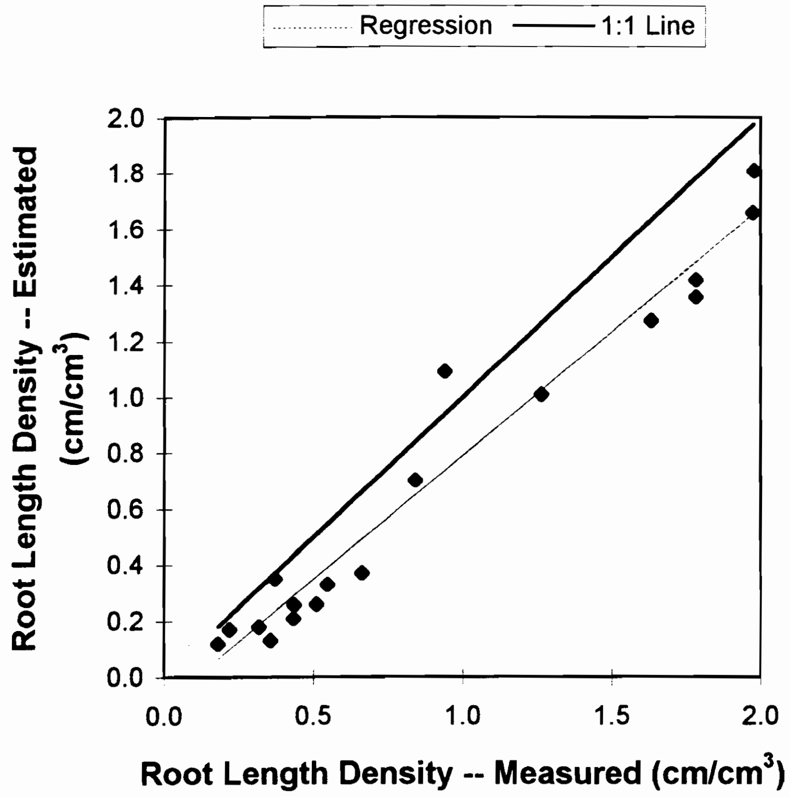

Figure 4.15. Relationship between estimated Root Length Density (cm/cm^3) and measured Root Length Density (cm/cm^3)

Table 4.6 shows the coefficients of the regression equation calculated from the relationship between estimated Root Length Density and measured Root Length Density. 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 is 0.959).

Table 4.6. Relationship between estimated Root Length Densities and measured Root Length Densities of bulk soil contained in soil cores.

Regression Coefficient(β_1)	Intercept(β_0)	R Square
0.8844	-0.0927	0.9589

4.3 Discussion

The Root Length Densities found in this research were comparable to published values. Fusseder (1983) found a maximal Rooting Density of 0.67 cm/cm^3 for a 6-weeks-old corn root system. Smika and Klute (1982) reported Root Length Density ranging from 0.4 to 2.6 cm/cm^3 depending on the time of sampling. Allmaras et al. (1975) found a Root Length Density of 0.43 cm/cm^3. The similarity between our Root Length Density value range and others indicates that our Root Length Density values are reasonable. Our Root Length Density distribution also follows the general trend. Generally Root Length Density decreases with increase in depth. Our results show that Root Length Densities at the near soil surface were always greater than deeper ones at any distance from the corn stem. Figure 4.16 shows the Root Length Density distribution trend according to sampling depth and distance from corn stem.

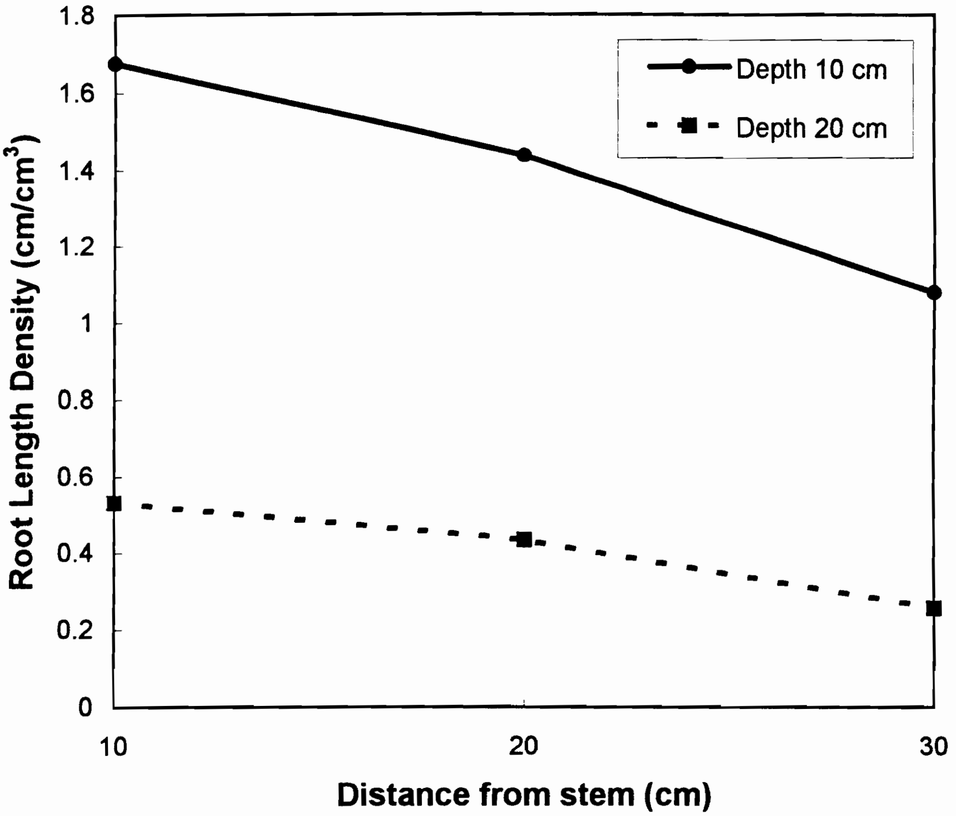

Figure 4.16. Root Length Density distribution according to depth and distance.

4.3.1 Roots Exposing

In the process of recording the roots-soil image, special care was taken for exposing roots at the soil profile. Several tools were tried to expose roots efficiently, but each had limitations. A hard plastic brush wiped out fine roots. A soft paint brush could not loosen soil particles. A thin metal brush smashed roots. Water spray removed soil particles and uncovered some roots. However, the loosened soil particles covered other roots. Furthermore, the soil-water mix made the environment unsuitable for using electronics. Using an air spray dried the roots. Combining several tools through several steps exposed roots clearly. Soil particles had to be loosened after the root-soil sample was cut because the cutting procedure hardened the soil planar face. It was impossible to differentiate roots from the soil at the hardened soil profile. After carefully loosening soil particles with a small driver, the loosened soil was removed by soft paint brush. Then, water was sprayed to prevent the roots from drying. When treating the soil-roots sample, plastic cap was put on the ends of the sample to prevent soil-crumbling. It was evident that roots could not be identified from the cut soil surface without these additional root- exposing steps.

4.3.2 Staining

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. The autofluorescence of corn roots is caused by the presence of special a compound called "Coumarin." The Coumarins have the characteristic fluorescence on ultraviolet (UV) irradiation, and there are several kinds of natural coumarins. Corn roots contain three kinds of natural coumarin: Coumarin, Scopoletin, and Umbelliferone (Murray et al., 1982). These three coumarins are responsible for the corn root's autofluorescence. This staining technique can be used to study other crops because most crop roots contain certain natural coumarins.

4.3.3 Length Measurement by Image Analysis

The Line Intersect method usually has lower accuracy and precision than Image Analysis method (Lebowitz, 1988). This problem is caused by the non-random placement of the root, wire, or thread samples, because the Line Intersection method assumes random placement of samples. To correct this problem, additional times of repositioning and scanning each sample are required (Lebowitz, 1988), involving additional time and labor. In contrast, in the Image Analysis method, the sample does not need to be repositioning because image thinning is an orientation invariant process. Therefore, Image Analysis is particularly suitable for measuring root length *in situ,* where a root system cannot be repositioned. Another advantage of using Image Analysis is that it can be used to measure other root parameters such as root surface area. Because roots are basically cylindrical objects, the root surface area can be calculated by multiplying root length, root diameter, and π (Lebowitz, 1988; Ottman and Timm, 1984; Smika and Klute, 1982).

4.3.4 Comparison with other Methods

The Minirhizotron and Core break methods are comparable techniques to our Image Analysis method. All methods reduce the time and labor required to determine Root Length Density by eliminating the root washing procedure. In fact, the former two techniques could estimate Root Length Density without washing roots from soil, but both techniques have some limitations.

Minirhizotron techniques also find a linear relationship between root length densities determined by minirhizotrons and those determined by soil sampling. However, estimated Root Length Densities from the minirhizotron technique were significantly lower than measured Root Length Densities from core sampling in the top layer of soil (Bragg et al., 1983; Gregory, 1979; Merrill et al., 1987; Upchurch and Ritchie, 1983; Vos and Groenwold, 1987). The reason for this problem has not been explained. It was not caused by light entering the tubes or improperly insulating the exposed portion of the tube, which might cause temperature effects (Samson and Sinclair, 1994). Considering the fact that more than 45 % of the Root Length Density was found with the core sampling technique in this layer (Samson and Sinclair, 1994), this problem is very serious. Because of this problem the correlation found by the minirhizotron technique were high only when the near surface depths data were excluded from calculation. Upchurch and Richie (1983) used depths greater than 20 cm to get a higher correlation coefficients. According to them the correlation near the soil surface was near zero or negative.

Another reason not to use minirhizotron counts for estimating Root Length Density is the higher values at the soil-tube interface than elsewhere. Since the growth of roots near the tube is affected by the presence of the tube, the estimated Root Length Density near the observation tube differs from the Root Length Density of bulk soil (Volkmar, 1993; Upchurch, 1987), perhaps because disturbance by the observation tube affects environmental conditions such as temperature and moisture in the soil close to the tube.

Differences in the physical condition of the soil can also affect the accuracy of minirhizotron estimates. Volkmar (1993) found a significant slope difference for different soil bulk densities when he computed the relationship between minirhizotron root counts and measured root length density.

Furthermore, to find a satisfactory correlation with bulk soil root length density, results from several tubes had to be averaged. Upchurch and Ritchie (1983) show that the variability of the data from individual minirhizotrons was so great that the results from a single tube have very little correlation with the bulk soil Root Length Densities. When the results of individual minirhizotrons were used, the correlation coefficients were 0.16 for all depths, and 0.39 for depths greater than 20 cm. When the mean of 8 minirhizotrons was used, the correlation coefficients were 0.35 and 0.81 for all depths and depths greater than 20 cm, respectively. Using the minirhizotron method, Volkmar (1993) found 0.54 ~ 0.83 of R square values from the regression equations between minirhizotron root counts against measured Root Length Densities.

The important advantage of the Root Length Density measurement method proposed in this research over the Core break method is that Image Analysis does not need separate calibration for different soils, crops, and dates. In principle, for the Core break method, the proportionality between root counts per area (RCA) and Root Length Density (RLD) is changed by the distribution of angles of root growth. If the root system is randomly distributed, the ratio RLD/RCA = 2.0 or β_1 is 0.5 (Lang and Melhuish, 1970). However, roots tend to have a prefered direction. A horizontal-preferred root system has greater RLD/RCA ratio than the theoretical value 2.0, such as reported by Bland (1989) 7.7 ($\beta_1 = 0.13$) for sorghum.

These problems, which hinder the use of the Minirhizotron or the Core break techniques were not found in Image Analysis. Rooting Intensities were highly correlated with Root Length Densities.

The agreement between estimated Root Length Density and measured Root Length Density for corn roots (Table 4.6) would suggest that Rooting Intensity of the soil core can be converted to estimates of Root Length Density in the soil. This conversion will reduce the time required for measuring root length, and it can eliminate the need for relatively severe destructive sampling methods.

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.

4.3.5 Efficiency of the Method

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. It include 10 min for taking the core sample, 5 min for preparing and recording the root image in the field, and 5 min for processing and estimating root length in the lab. In contrast, the time required for the conventional method to process the soil was about 50 min (Bohm et al., 1977). This rate of measurement is nearly 2.5 fold greater than previous methods in the literature.

Image analysis thus represents both a time saving and the possibility to collect more root information during a season.

V. Summary and Conclusions

The roots research is important for finding plant-soil relationships, managing soil effectively, studying nutrients and water extraction, and creating a soil quality index. However, studying roots requires a great deal of time and labor, because of inefficient root measurement methods and the necessity for washing the roots. Thus, effective root research required a method for reducing these time and labor requirements. Choosing rapidly measurable root parameters, using efficient measurement methods, and eliminating the washing procedure are keys to the reduction of time and labor. To achieve this reduction, root parameters were reviewed, and root length was selected as a adequate root parameter for rapid determination. Also, Image Analysis method is proposed to eliminate the need for root washing.

This method 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 take root-soil samples from a corn field. The core sample was cut by knife and the roots were exposed by careful handling. The root-soil sample was put into a box to protect from sunlight. The exposed roots faced up to the camcorder. Long-wave ultraviolet light illuminated the root-soil sample to brighten the live roots selectively. The contrast-enhanced image was recorded on the camcorder. The recorded root image was digitized by frame grabber and turned into a gray scale digital image. The roots were differentiated from the background by thresholding the gray scale image and 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 to 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 roots sample. 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 with a CCD camera in lab. The root length density was calculated by dividing the root length as measured from above steps by the volume of core sample. The Rooting Intensity and root length density value were paired to find a linear relationship.

Before the image analysis method was applied to a field experiment, the length measurement system was evaluated by comparing actual lengths with measured lengths of sewing thread. The measured and actual thread lengths were highly correlated.

Based on these results, the following conclusions are drawn.

1. The proposed Image Analysis-based root length density measurement procedure was effective in reducing the time and labor required for root study. By choosing the right components - UV light-based root staining, core sampling, image analysis, rooting intensity - and composing them, rapid determination of root length density was possible.

2. The thinning algorithm-based Image Analysis system can be used for some research where the accurate length of washed roots is needed. The image analysis-based root length measuring system was more precise and faster than the Line Intersection system.

VI. Recommendations for Future Work

Based on the information acquired from this study, the following suggestions are made for future study:

At the early stage of this study, rapid estimation of root length density which takes root images from a soil profile produced by tractor ploughing was considered. This idea turned out to be impractical. Although a sharp and thin knife was used, the resulting cut soil surface looked like plastered wall. Without the careful roots exposing procedure described in this paper, roots may not discernible from the soil matrix. In addition, the dull plough may crush and tear out roots. To acquire reliable root data from the soil cut surface, an appropriate root exposing procedure should be considered.

The technique has been applied directly to field measurements by capturing images with a portable video camera and video recorder. However, the images were analyzed in the lab because the image processing system was too large to take to the field. Its size prevents assessing root information at the site. To collect root information quickly, the analysis should be done in the field. By using a portable computer and a small frame grabber that can fit on the parallel port, root information can be extracted in the field without returning to the lab to analyze the image data. Small digitizing hardware can be found in the market.

While the validity of an Image Analysis-based root length density measurement method was evaluated in this research, more extensive experiments should be undertaken to increase the reliability of this measurement method. It should be applied to other crops. It can also be used to compare different soil management procedures.

The proposed method can be made more usable in terms of following sense. Information concerning early-season corn root distribution can enable the farmer to position fertilizer to maximize root interception. By assessing the root length density, this system can examine tillage systems, or the effect of wheel traffic on crop production.

The Image Analysis method can be used to estimate root length of white roots in experiments with plant seedlings grown in solution culture. Because of the nondestructive characteristic of this method, it is useful in experiments studying the effects of root-zone temperature, substrate fertility, and plant-growth regulating chemicals on dynamic root system development. The growth of vegetables can be monitored as well.

VII. List of References

Adams, M.B., R.J. Luxmoore, W.M. Post, and D.L. DeAngelis. 1988. Quick estimates of root length, using a video image analyzer. *Canadian Journal of Forest Research* 19:335-340.

Allmaras, R.R., W.W. Nelson, and W.B. Voorhees. 1975. Soybean and corn rooting in Southwestern Minnesota. Pp. 771-777 *In: Root Distributions and Related Water Inflow*. Proceedings of Soil Science Society of America 39. Madison, Wisconsin; Soil Science Society of America.

Arkin, G.F. and H.M. Taylor, eds. 1981. Modifying the root environment to reduce crop stress. St. Joseph, Michigan: American Society of Agricultural Engineers.

Arshad, M.A. and G.M. Coen. 1992. Characterization of soil quality: Physical and chemical criteria. *American Journal of Alternative Agriculture* 7(1-2):25-31.

Baldwin, J.P., P.B. Tinker, and F.H.C. Marriott. 1971. The measurement and distribution of onion roots in the field and the laboratory. *Journal of Applied Ecology* 8:543-554.

Baxes, Gregory A. 1988. *Digital Image Processing*. Denver, Colorado: Cascade Press.

Bennie, A.T.P., H.M. Taylor, and P.G. Georgen. 1987. An assessment of the core-break method for estimating rooting density of different crops in the field. *Soil Tillage Research* 9:347-353.

Bhar, D.S., G.F. Mason, and R.J. Hilton. 1970. In situ observations on plum root growth. *Journal of the American Society for Horticultural Science* 95(2):237-239.

Bland, W.L. 1989. Estimating root length density by the core-break method. *Soil Science Society of America Journal* 53:1595-1597.

Bland, W.L. 1991. Root length density from core-break observations: Sources of error. Pp.565-569 *In: Plant Roots and Their Environment*, eds. B. L. McMichael and H. Persson. Amsterdam: Elsevier Science Publishers.

Bland, W.L. and W.A. Dugas. 1988. Root length density from minirhizotron observations. *Agronomy Journal* 80:271-274.

Bohm, W. 1979. *Methods of Studying Root Systems*. Ecological Studies, vol. 33. Berlin Heidelberg, Germany: Springer-Verlag.

Bohm, W., H. Maduakor, and H.M. Taylor. 1977. Comparison of five methods for characterizing soybean rooting density and development. *Agronomy Journal* 69:415-418.

Bohm, W. 1976. In situ estimation of root length at natural soil profiles. *Journal of Agricultural Science* 87:365-368.

Bragg, P.L., G Govi, and R.Q. Cannell. 1983. A comparison of methods, including angled and vertical minirhizotrons, for studying root growth and distribution in a spring oat crop. *Plant and Soil* 73:435-440.

Brar, G.S., A.G. Matches, H.M. Taylor, B.L. McMichael, and J.F. Gomez. 1990. The methods for characterizing rooting depth of forge-legume seedlings in the field. *Crop Science* 30:413-417.

Buckland, S.T., C.D. Campbell, L.A. Mackie-Dawson, G.W. Horgan, and E.I. Duff. 1993. A Method for counting roots observed in minirhizotrons and their theoretical conversion to root length density. *Plant and Soil* 153:1-9.

Bui, Elisabeth N. and James E. Box, Jr. 1993. Growing corn root effect on interrill soil erosion. *Soil Science Society of America Journal* 57:1066-1070.

Casarin, M., S. Jacquey, A. Fouere, and Ph. Girardin. 1991. Digital picture processing applied to the evaluation of plant root dynamics. Pp. 570-575 *In: Plant Roots and*

Their Environment. B.L. McMichael and H. Persson, eds. Developments in Agricultural and Managed-Forest Ecology 24. Amsterdam: Elsevier Science Publishers.

Commins, P.J., A.B. McBratney, and A.J. Koppi. 1991. Development of a technique for the measurement of root geometry in the soil using resin-impregnated blocks and image analysis. *Journal of Soil Science* 42:237-250.

Cunningham, M., M.B. Adams, R.J. Luxmoore, W.M. Post, and D.L. DeAngelis. 1989. Quick estimates of root length, using a video image analyzer. *Canidian Journal of Forest Research* 19:335-340.

Drew, M.C. and L.R. Saker. 1980. Assessment of rapid method, using soil cores, for estimating the amount and distribution of crop roots in the field. *Plant Soil* 55:297-305.

Elkins, C.B., R.L. Haaland, and C.S. Hoveland. 1977. Grass roots as a tool for penetrating soil hardpans and increasing crop yields. Pp. 21-26 *In: Proceedings of the Southern Pasture and Forage Crop Improvement Conference*. Auburn, Alabama, 12-14 April. New Orleans, Louisiana: Agricultural Research Service.

Ferguson, J.C. and A.J.M. Smucker. 1989. Modifications of the minirhizotron video camera system for measuring spatial and temporal root dynamics. *Soil Science Society of America Journal* 53:1601-1605.

Fusseder, A. 1983. A method for measuring length, spatial distribution and distances of living roots in situ. *Plant and Soil* 73(3):441-445.

Gale, M.R., D.F. Grigal, and R.B. Harding. 1991. Soil productivity index: Predictions of site quality for white spruce plantations. *Soil Science Society of America Journal* 55:1701-1708.

Gijsman, A.J., J. Floris, M. Van Noordwijk, and G. Brouwer. 1991. An inflatable minirhizotron system for root observations with improved soil-tube contact. *Plant and Soil* 134:261-269.

Girardin, Ph., R. Trendel, S. Jacquey, M. Casarin, M. Goss, and A. Fouere. 1991. Comparison of situ root observations with rhizoscope, video recording and endoscope. Pp. 603-607 *In: Plant Roots and Their Environment.* B.L. McMichael and H. Persson, eds. Developments in Agricultural and Managed-Forest Ecology 24. Amsterdam: Elsevier Science Publishers.

Glasbey, C.A., G.W. Horgan, and J.F. Dargyshire. 1991. Image analysis and three-dimensional modelling of pore in soil aggregates. *Journal of Soil Science* 42:479-486.

Glenn, D.M., M.W. Brown, and F. Takeda. 1987. Statistical analysis of root count data from minirhizotrons. Pp. 81-87 *In: Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics,* ed. H.M. Taylor. ASA Special Publication No. 50. Madison, Wisconsin: American Society of Agronomy.

Gonzalez, Rafael C., and Richard E. Woods. 1992. *Digital Image Processing.* Reading, Massachusetts: Addison-Wesley Publishing Company.

Granatstein, D. and D.F. Bezducek. 1992. The need for a soil quality index: Local and regional perspectives. *American Journal of Alternative Agriculture* 7(1-2):12-16.

Gregory, P. J. 1979. A periscope method for observing root growth and distribution in field soil. *Journal of Experimental Botany* 30:205-214.

Haberern, J. 1992. Coming full circle - The new emphasis on soil quality. *American Journal of Alternative Agriculture* 7(1-2):25-31.

Harris, Grant A. and Gaylon S. Campbell. 1989. Automated quantification of roots using a simple image analyzer. *Agronomy Journal* 81:935-938.

Heeraman, D.A., P.H. Crown, and N.G. Juma. 1993. A color composite technique for detecting root dynamics of barley(*Hordeum vulgare* L.) from minirhizotron images. *Plant and Soil* 157:275-287.

Karlen, D.L., N.S. Eash, and P.W. Unger. 1992. Soil and crop management effects on soil quality indicators. *American Journal of Alternative Agriculture* 7(1-2):48-55.

Kaspar, T.C., H.M. Taylor, and R.M. Shibles. 1984. Taproot-elongation rates of soybean cultivars in the glasshouse and their relation to field rooting depth. *Crop Science* 24(September-October):916-920.

Klepper, B., H.M. Taylor, M.G. Huck, and E.L. Ficus. 1973. Water relations and growth of cotton in drying soil. *Agronomy Journal* 65:307-310.

Koppi, A.J. and A.B. McBratney. 1991. A basis for soil mesomorphological analysis. *Journal of Soil Science* 42:139-146.

Koppi, A.J., D.C. McKenzie, J.T. Douglas. 1994. Images of soil structure for illustration of machinery effects. *Journal of Agricultural Engineering Research* 57:67-72.

Lang, A.R.G. 1972. Lengths and diameters of plant roots in non-random populations by analysis plane surfaces: A correction. *Biometrics* 28:625-627.

Lang, A.R.G. and F.M. Melhuish. 1970. Lengths and diameters of plant roots in non-random populations by analysis of plane surfaces. *Biometrics* 26:421-431.

Lebowitz, R.J. 1988. Digital image analysis measurement of root length and diameter. *Environmental and Experimental Botany* 28:267-273.

Lindstrom, M.J., T.E. Schumacher, A.J. Jones, and C. Gantzer. 1992. Productivity index model comparison for selected soils in North Central United States. *Journal of Soil and Water Conservation* 47(6):491-494.

Matyac, C.A., G.P. Cofer, J.E. Bailey, and G.A. Johnson. 1989. In situ observation of root-gall formation using nuclear magnetic resonance imaging. *Journal of Nematology* 21(1):131-134.

McLean, M., G.S. Howell, and A.J.M. Smucker. 1992. A minirhizotron system for *in situ* root observation studies of seyval grapevines. *American Journal of Enology and Viticulture* 43(1):87-89.

McMichael, B.L., D.R. Upchurch, and H.M. Taylor. 1992. Transparent wall techniques for studying root growth and function in soil. *Journal of Plant Nutrition* 15(6-7):753-762.

Melhuish, F.M. and A.R.G. Lang. 1968. Quantitative studies of roots in soil. I. Length and diameters of cotton roots in an clay-loam soil by analysis of surface-ground blocks of resin-impregnated soil. *Soil Science* 106:16-22.

Merrill, S.D., E.J. Doering, and G.A. Reichman. 1987. Application of a minirhizotron with flexible, pressurized walls to a study of corn root growth. Pp. 131-143 *In: Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics.* ed. H.M. Taylor. Special Publication No. 50. Madison, Wisconsin: American Society of Agronomy.

Meyer, W.S. and H.D. Barrs. 1985. Non-destructive measurement of wheat roots in large undisturbed and repacked clay soil cores. *Plant and Soil* 85:237-247.

Murray, R.D.H., J. Mendez, and S.A. Brown. 1982. *The Natural Coumarins*. New York; John Wiley & Sons Ltd.

Myler, Harley R. and Arthur R. Weeks. 1993. *Computer Imaging Recipes in C*. Englewood Cliffs, New Jersey: P T R Prentice Hall.

Ottman, M.J. and H. Timm. 1984. Measurement of viable plant roots with the image analyzing computer. *Agronomy Journal* 76:1018-1020.

Oussible, M., R.K. Crookston, and W.E. Larson. 1992. Subsurface compaction reduces the root and shoot growth and grain yield of wheat. *Agronomy Journal* 84:34-38.

Pallant, Eric, Richard A. Holmgren, George E. Schuler, Kimberly L. McCracken, and Barbara Drbal. 1993. Using a fine root extraction device to quantify small diameter corn roots (>=0.025 mm) in field soils. *Plant and Soil* 153:273-279.

Pan, W.L. and R.P. Bolton. 1991. Root quantification by edge discrimination using a desktop scanner. *Agronomy Journal* 83:1047-1052.

Parr, J.F., R.I. Paperndick, S.B. Hornick, and R.E. Meyer. 1992. Soil quality: Attributes and relationship to alternative and sustainable agriculture. *American Journal of Alternative Agriculture* 7(1-2):5-11.

Poincelot, Raymond P. 1986. *Toward a More Sustainable Agriculture*. Westport, Connecticut: AVI Publishing Company, Inc.

Pratt, William K. 1991. *Digital Image Processing*. New York: John Wiley & Sons, Inc.

Ralph, A. Olsen, Ralph B. Clark, and Jesse H. Bennett. 1981. The enhancement of soil fertility by plant root. *American Scientist* 69:378-384.

Reicosky, D.C., R.J. Millington, and D.B. Peters. 1970. A comparison of three methods for estimating root length. *Agronomy Journal* 62:451-453.

Reid, J.B. and M.J. Goss. 1981. Effect of living roots of different plant species on the aggregate stability of two arable soils. *Journal of Soil Science* 32:521-541.

Rendig, V.V. and Howard M. Taylor. 1989. *Principles of Soil-Plant Interrelationships*. New York: MacGraw Hill.

Ruark, G.A. and J.G. Bockheim. 1988. Digital image analysis applied to soil profiles for estimating tree root biomass. *Soil Science* 146(2):119-123.

Samson, Benjamin K. and Thomas R. Sinclair. 1994. Soil core and minirhizotron comparison for the determination of root length density. *Plant and Soil* 161:225-232.

Sanders, J.L. and D.A. Brown. 1978. A new fibre optic technique for measuring root growth of soybeans under field conditions. *Agronomy Journal* 70:1073-1076.

Shuman, L.M., E.L. Ramseur, and D.O. Wilson. 1993. Video image method compared to a hand method for determining root lengths. *Journal of Plant Nutrition* 16(4):563-571.

Simmons, G.L. and P.E. Pope. 1988. Development of a root growth model for yellow-poplar and sweetgum seedlings grown in compacted soil. *Canadian Journal of Forest Research* 18: 728-732.

Smika, D.E. and A. Klute. 1982. Surface area measurement of corn root system. *Agronomy Journal* 74:1091-1093.

Smit, A.L., J.F.C.M. Sprangers, P.W. Sablik, and J. Groenwold. 1994. Automated measurement of root length with a three-dimensional high-resolution scanner and image analysis. *Plant and Soil* 158:145-149.

Smucker, Alvin. 1994. Indicators of sustainability. *E-mail to listserver Soils-L*, <smucker@ajms.pss.msu.edu>.

Smucker, A.J.M., J.C. Ferguson, W.P. DeBruyn, R.K. Belford, and J.T. Ritchie. 1987. Image analysis of video-recorded plant root systems. Pp. 67-80 *In: Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics.* ed. H.M. Taylor. Special Publication No. 50. Madison, Wisconsin: American Society of Agronomy.

Smucker, A.J.M., S.L. McBurney, and A.K. Srivastara. 1982. Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. *Agronomy Journal* 74:500-503.

Spomer, L. Art and M.A.L. Smith. 1989. Image analysis morphometric measurement for tissue water status and other determinations. *Agronomy Journal* 81:906-910.

Stoskopf, Neal C. 1981. *Understanding Crop Production*. Englewood Cliffs, New Jersey: Prentice-Hall, Inc.

Stutte, G.W. 1990. Analysis of video images using an interactive image capture and analysis system. *HortScience* 25(6):695-697.

Tagliavini, M., L.J. Veto, and N.E. Looney. 1993. Measuring root surface area and mean root diameter of peach seedlings by digital image analysis. *Hortscience* 28(11):1129-1130.

Taylor, H.M., ed. 1987. *Minirhizotron Observation Tubes: Methods and Applications for Measuting Rhizosphere Dynamics*. ASA Special Publication No. 50. Madison, Wisconsin: American Society of Agronomy.

Taylor, H.M., M.G. Huck, Betty Klepper, and Z.F. Lund. 1970. Measurement of soil-grown roots in a rhizotron. *Agronomy Journal* 62:807-809.

Taylor, H.M., D.R. Upchurch, and B.L. McMichael. 1990. Applications and limitations of rhizotrons and minirhizotrons for root studies. *Plant and Soil* 129:29-35.

Taylor, H.M., D.R. Upchurch, J.M. Brown, and H.H. Rogers. 1991. Some methods of root investigations. Pp. 553-564 *In: Developments in Agricultural Managed-Forest Ecology*, vol. 24. Amsterdam: Elsevier Scientific Publishing Company.

Troeh, Frederic R., J. Arthur Hobbs, and Roy L. Donahle. 1991. *Soil and Water Conservation*. Englewood Cliffs, New Jersey: Prentice-Hall.

Upchurch, D.R.. 1987. Conversion of minirhizotron-root intersections to root length density. Pp. 51-65 *In: Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics*, ed. H.M. Taylor. ASA Special Publication No. 50. Madison, Wisconsin: American Society of Agronomy.

Upchurch, D.R. and J.T. Ritchie. 1983. Root observations using a video recording system in minirhizotrons. *Agronomy Journal* 75:1009-1015.

van de Vooren, J.G. 1994. Measuring the size of pods french beans with image analysis. ASAE paper No. 943508. St. Joseph, Michigan: American Society of Agricultural Engineers.

van Noordwijk, M. and G. Brouwer. 1991. Review of Quantitative root length data in agriculture. Pp. 515-525 *In: Plant Roots and Their Environment*. B.L. McMichael and H. Persson, eds. Developments in Agricultural and Managed-Forest Ecology 24. Amsterdam: Elsevier Science Publishers.

Virginia, R.A. and W.M. Jarrell. 1987. Approaches for studying the function of deep root systems. Pp107-127 *In: Plant Response to Stress: Functional Analysis in Mediterranean Ecosystems*, ed. J.D. Tenhunen et al. NATO ASI Series, vol. G15. Sezimbra, Portugal: NATO Advanced Research Workshop on Plant Response to Stress.

Visser, Suzanne and Dennis Parkinson. 1992. Soil biological criteria as indicators of soil quality: Soil microorganisms. *American Journal of Alternative Agriculture* 7(1-2):33-37.

Volkmar, K.M. 1993. A comparison of minirhizotron techniques for estimating root length density in soils of different bulk densities. *Plant and Soil* 157:239-245.

Voorhees, W.B., V.A. Carlson, and E.A. Hallauer. 1980. Root length measurement with a computer-controlled digital scnning micro-densitometer. *Agronomy Journal* 72:847-851.

Vos, J. and J. Groenwold. 1983. Estimation of root densities by observation tubes and endoscope. *Plant and Soil* 74:295-300.

Vos, J. and J. Groenwold. 1987. The relation between root growth along observation tubes and in bulk soil. Pp. 39-49 *In: Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics*. ed. H.M. Taylor. ASA Special Publication No. 50. Madison, Wisconsin: American Society of Agronomy.

Waldron, L.J. 1977. The shear resistance of root-permeated homogeneous and stratified soil. *Soil Science Society of America Journal* 41:843-849.

Yanuka, M. and D.E. Elrick. 1985. Application of microcomputer-based image digitization in soil and crop sciences. *Computers and Electronics in Agriculture* 1:59-73.

Vita

I was born in Taebak, South Korea, on April 7, 1966. I was raised in a loving family; two parents and one sister. I received a B.S. in Agricultural Engineering from Seoul National University in 1988. After receiving a M.S. in Agricultural Engineering from the same university in 1990, I served in the army as a reserve officer's candidate. I returned to the university and worked as an administrative assistant for one year. In Fall 1992 I enrolled at VPI in Agricultural Engineering Department (now it is Biological Systems Engineering) as a Korean Government Fellowship student.

Giyoung Kim
Blacksburg, Virginia

Kim Giyoung