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Plant roots and their environment
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SOME METHODS OF ROOT INVESTIGATIONS

H.M. Taylor[1], D.R. Upchurch[2], J.M. Brown[3], and H.H. Rogers[4]

1. Agronomy, Horticulture and Entomology Department, Texas Tech University, Lubbock, Texas (USA).
2. USDA-ARS, Lubbock, Texas (USA).
3. Duke University Medical School, Durham, North Carolina (USA).
4. USDA-ARS, Auburn, Alabama (USA).

ABSTRACT

This article discusses 6 methods of root investigations. At least one of the authors has used each technique during the previous 10 years. The 6 techniques are a simple spade, the core-break, mini-rhizotrons, computer-assisted tomography, neutron radiography, and nuclear magnetic resonance imaging. Some advantages and disadvantages of each technique are discussed.

INTRODUCTION

The study of biological organisms always is difficult because of the complex interactions that occur. This difficulty is increased substantially by the environment in which the plant's root system exists. The root system is hidden from direct observation by the soil matrix. As a result of the shielding effect of the soil matrix, most measurements of the root system are either disruptive or totally destructive to the roots being studied and to their immediate environment. When the root environment is changed in order to allow observations of the roots, a question always arises about the impact of the measurement on the results of the observation.

A further complication involved in studying roots arises from spatial and temporal variability of the roots and of the soil system. Roots are not uniformly distributed in the soil with depth, distance from the crown or with time. Soil properties also are not uniform at the scale of an individual root or with time. We should not expect the root system to grow or to function uniformly throughout its lifetime.

The study of root and soil dynamics involves many scientific disciplines, each requiring specialized research procedures. We will discuss six methods of studying roots, thereby excluding from discussion many other useful methods. Our rationale for including these six methods is that at least one of the authors has used each of these techniques during the past 10 years. We thus feel confident in discussing their advantages and disadvantages. Böhm (1979) has an excellent discussion of many other methods.

1. EXCAVATION WITH A SPADE

Root system studies in field environments are so tedious and time consuming that one should use the simplest possible procedure that will provide the information that is needed. A vertical slice of the plowed field may be sufficient to detect a pressure pan (Figure 1) or the presence of nematodes on a root system. Several slices can be taken and the roots observed within those slices within a few minutes. This procedure provides qualitative answers to some specific questions at very little cost. However, the technique destroys the roots being studied.

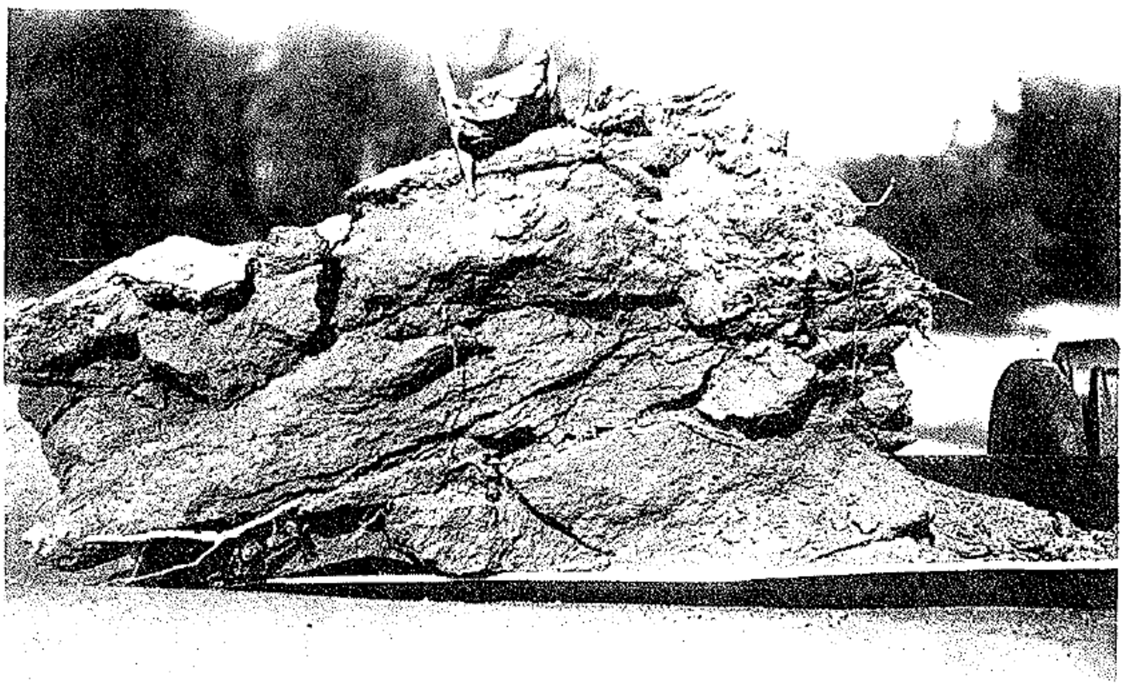

Fig. 1. A spade is used to dig through the plow layer of a sandy soil. Roots of a cowpea (Vigna sinensis) plant are growing along horizontally oriented soil cracks.

2. CORE-BREAK TECHNIQUE

Many systems have been described for collecting soil cores. Basically, soil is removed from a cylindrical cavity usually by inserting then extracting a sampling tube to the desired depth. Diameters of the cores vary from 25 to 100 mm with a 50 mm diameter being the most common.

All soil coring techniques have advantages and disadvantages when compared to other methods for determining root characteristics (Böhm, 1979). In addition, the core-break techniques has advantages and disadvantages when compared to other soil coring techniques.

Soil coring techniques have the primary advantage of reduced time and labor inputs over the framed monolith methods (Böhm et al., 1977). For example, they (1977) estimated that 1560 person minutes were required to obtain one large monolith, refill the hole, wash soil from the root system, clean debris from the roots and to machine count root length in depth increments. It took about 1/3 as long to take a soil core with a coring machine, to clean the soil and debris from the roots and measure them. The actual soil coring process required only a small proportion of the time because cleaning debris from the root sample was the major time consumer in both techniques.

A wide range of tools is available for collecting intact soil cores. These tools range in complexity from simple hand driven tools to complex hydraulic coring devices, such as the one marketed by Giddings Machine Company, Fort Collins, Colorado.

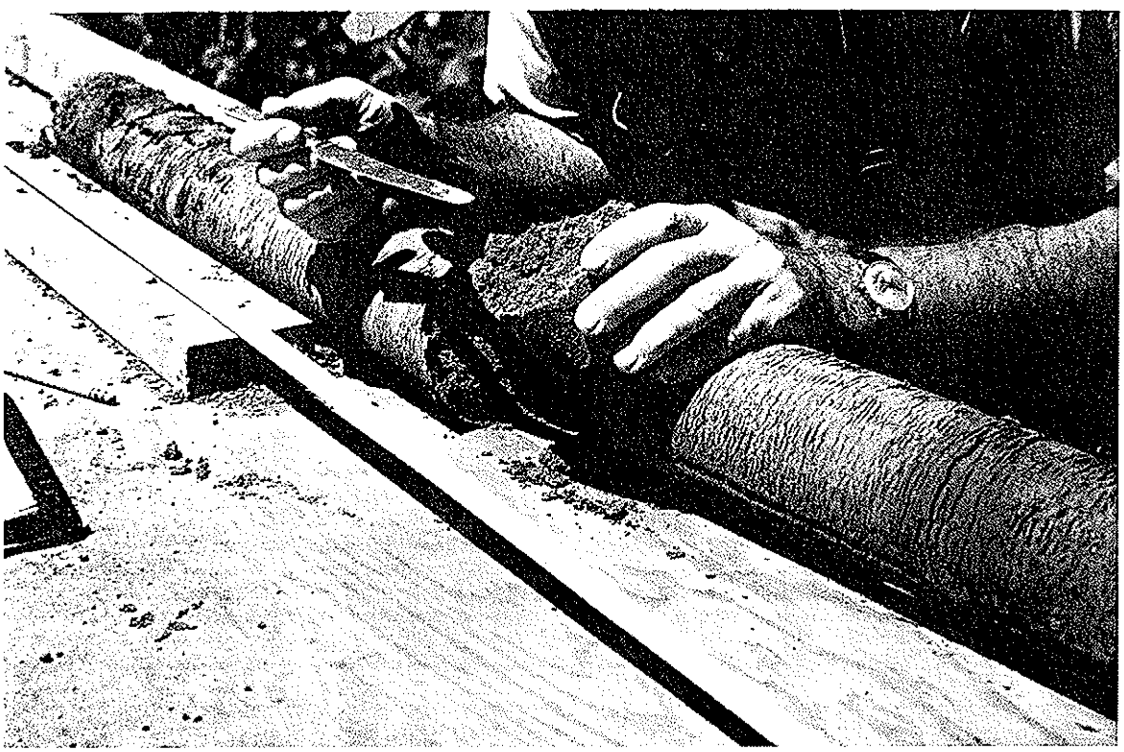

Fig. 2. A technician is counting the number of roots that project from the 2 broken surfaces of a soil core in the core-break technique.

A large number of cores can be collected in a relatively short period of time without severe damage to the plot or plants. Results from coring have a large variance, which limits usefullness of soil coring in determining small differences in rooting. Soil cores are difficult to obtain from stony soils, very high-strength soils or soils that crumble easily. Soil coring is not adapted to studies of root morphology because only a limited part of the root system is sampled. Even with these limitations, soil coring is a frequently used technique.

Drew & Saker (1980) described a core-break modification of the soil coring technique. They (1980) obtained a coherent soil core, which they broke laterally at specified depths in the soil profile. They then counted the roots that projected from both faces of the horizontal break (Fig. 2). Finally, they developed a relationship to obtain root length density form the number of roots that project from the two faces of the broken soil core.

Bennie et al. (1987) and Bland (1988) have investigated some of the problems associated with using the core-break method to obtain a measure of root length density.

Bennie et al. (1987) found that the slopes of equations describing relationships between root length density and the number of roots that projected from the broken faces varied with crop, soil profile characteristics and time. The slopes of these relationships changed primarily because the relative proportions of vertically and horizontally growing roots changed.

Human error also affects usefullness of the core-break technique (Bland, 1988). It is very easy to overlook small roots that break exactly at the soil break surface. It also is difficult to obtain a coherent core in non-cohesive or stony soils.

Despite the problems associated with soil coring in general and the core-break technique in particular, Bennie et al. (1987) recommended the core-break method for researchers and agricultural advisors who want to use a rapid method to quantify root development under various soil tillage treatments or practices. They found that it is possible to make several observations in a field or test plot in a relatively short period of time with minimum destruction if soil conditions are favorable for easy coring. Bennie (pers. comm., 1987) found that one extra person added to a 3-person crew that is determining gravimetric water contents could also determine rooting densities without an appreciable increase in sampling times.

3. MINI-RHIZOTRONS

In 1937, Bates proposed the ideas that roots could be observed through the walls of clear cylinders inserted into the soil profile. He used a mirror and stick arrangement to view roots through the walls of the cylinder. Waddington (1971) used a fiberoptic scope in place of the mirror and stick and then Sanders & Brown (1978) attached a camera to a fiberoptic scope to permanently record the image. Dyer & Brown (1980) replaced the camera with a black and white video camera then Upchurch & Ritchie (1984) used a color video camera to record root and soil images. Current equipment and procedures used in this technique are described by Brown & Upchurch (1987).

The current technique involves installing clear plastic tubes a few days after the crop is

Figure 3. A technician is examining the T.V. monitor of a color video camera system for minirhizotrons.

planted. The tubes, marked at predetermined depths, are installed at 30 degrees from the vertical to prevent roots from following the soil-tube interface downward. A video camera, mounted on a rigid handle, is lowered into the clear tube at a slow rate. Output from the camera is observed through a video monitor mounted above ground (Fig. 3).

Various techniques have been used to convert the root observations at the soil-tube interface to root length density. Upchurch (1987) outlines several procedures for converting the root observations at the soil-tube interface to root length densities.

Upchurch & Ritchie (1983) suggested that a minimum of 8 tubes was necessary to accurately estimate rooting in an apparently uniform plot. Each tube required 30 to 45 minutes to install in the profile but routine observation of roots from the tube required about 10 to 15 minutes by two experienced individuals. The 1989 price is about U.S. $15,000 for the commercially available equipment to observe and record video pictures of the root using the minirhizotron technique. A more complete review of the minirhizotron technique is available in a symposium publication (Taylor, 1987).

4. COMPUTER-ASSISTED TOMOGRAPHY

Medical technology has been applied to the imaging of root systems located in soil. One of these techniques is that of computer-assisted tomography; the CAT scanner (Hainsworth & Aylmore, 1983). At least two different radiation sources, X-ray and gamma rays, are being used to measure the density of a matrix and to infer the distribution of water in that matrix. Because living plant material has a high concentration of water, roots can be seen readily in the generated image (Fig. 4).

Briefly, the sample under observation is placed in a fixed position between a radiation source and a detector. The radiation source and detector, which are fixed at a specified distance apart, travel a 360 degree circular path around the stationary object. The radiation beam passes through the sample and the transmitted radiation is measured by the detector. Upon reaching the detector, the attenuated radiation is converted to electrical impulses that are integrated by a computer then reconstructed into an image representing the internal structure of the sample. See Brown et al. (1987) for a more complete description of the CAT procedure.

At the present time, the primary limitations to the use of the CAT scanners in root studies are expense, resolution and availability of the equipment. Cost of the equipment can range into millions of dollars, depending on the specific requirement for resolution. Increased sample size results in lowered resolution, which is on the order of a few millimeters when a full root system is being investigated. When smaller samples are used, the resolution can be on the order of 0.006 kg kg^{-1} over distances of 1.5 mm (Hainsworth & Aylmore, 1986). The ability of the radiation to penetrate the soil matrix is related to energy limitations. As the matrix thickness increases, the degree of attenuation increases and significant scattering can occur, which results in lowered resolution.

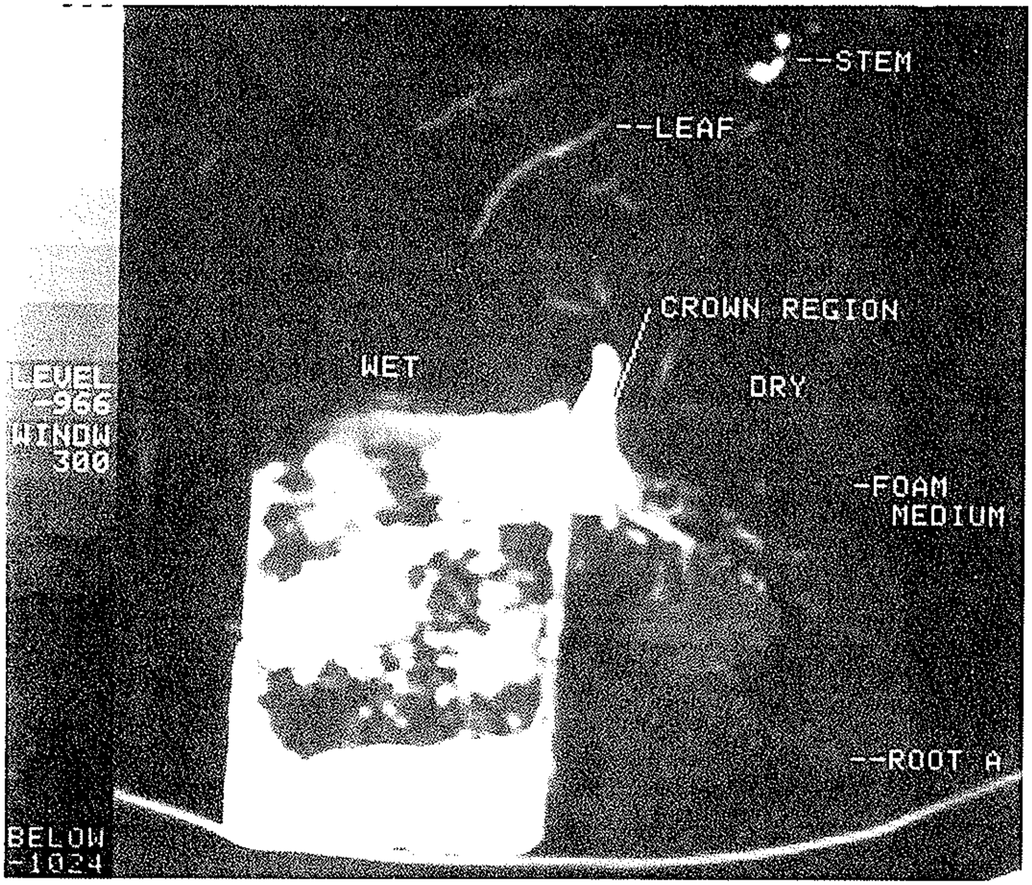

Fig. 4. A CAT scan of an experiment conducted by J.M. Brown and co-workers. In the experiment, a chrysanthemum root system was established from a cutting that was split at the base and rooted into two foam blocks. After the cutting was established, water was withheld from one side for 30 days while the other side was watered normally. Water contents in the foam on the dry side indicated that water was transferred from the wet to the dry side through the root system.

CAT scanners usually are located in major medical facilities. Technologists there often are reluctant to allow soil to be introduced into such a sanitized environment. This fact results in a problem of equipment accessability.

Despite these cost, resolution and accessability problems, CAT scanners have been used in at least four investigations on the distribution of water in various media (Hainsworth & Aylmore, 1983, 1986; Crestana et al., 1985; Brown et al., 1987).

5. NEUTRON RADIOGRAPHY

Root growth in soil also has been investigated using neutron radiography (Couchat & Moutonnet, 1974; Willatt & Struss, 1980; Willatt et al. 1978; Taylor & Willatt, 1983).

Roots in soil are locally high concentrations of water. Concentrations of water in roots effectively removes more neutrons from a collimated beam than does moist soil. The beam produces an image on a transfer screen, which can be further transferred to X-ray film. The image of the roots can be seen in contrast to the soil on the processed film (Fig. 5). Images can be seen readily up to soil thickness of about 5 cm. If soil thickness is lessened to 2 cm, resolution of the root diameter can be about 0.05 mm (Taylor & Willatt, 1983).

Safety for the investigators and accessability to a neutron source are the principal

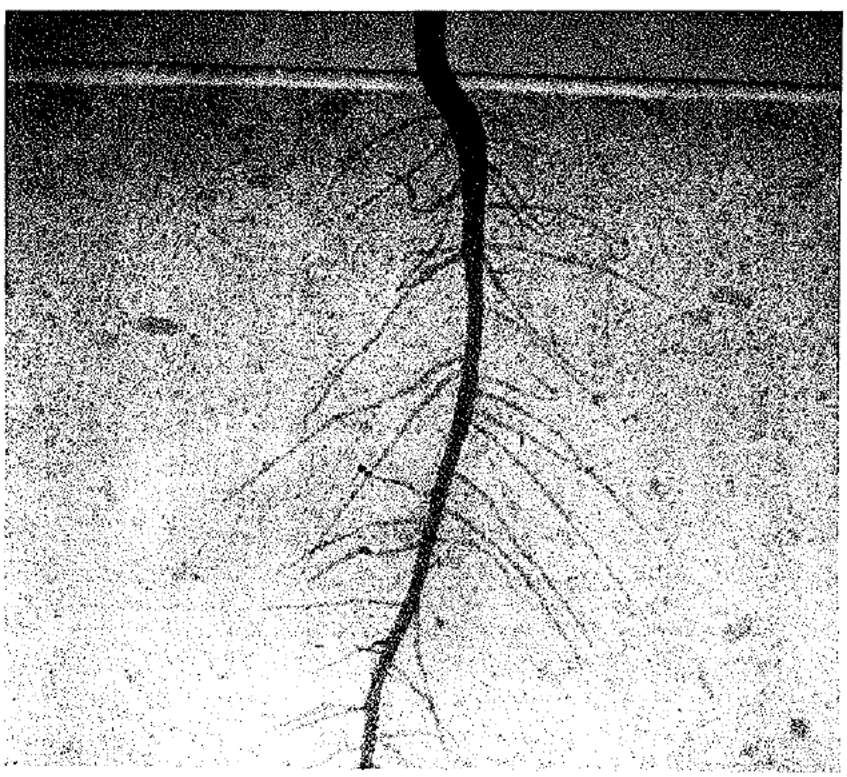

Fig. 5. Neutron radiograph of a bean (Phaseolus multiflorus) root system 7 days after planting in a soil that was 02% by weight of sand. The container for the soil was 150 X 150 X 25 mm. (Photo courtesy of S.T. Sillatt, La Trobe University).

problems associated with neutron radiography. There is a radioactive hazard associated with the beam because a beam strength of about 1.4 X 10^6 neutrons cm^{-2} sec^{-1} is required for precision measurements (Taylor & Willatt, 1983). In the USA, there are fewer nuclear reactors available to root physiologists than were available 10 years ago so the accessability problem has increased while equipment for other techniques has become more available.

6. NUCLEAR MAGNETIC RESONANCE IMAGING

Nuclear magnetic resonance imaging (NMR) is a nondestructive and non-invasive clinical technique used to image the internal anatomy of plants and animals. In NMR imaging, a sample is placed in a strong magnetic field, and a sequence of radio frequency (rf) pulses and magnetic field gradients is used to localize spatially (i.e. image) the concentration and

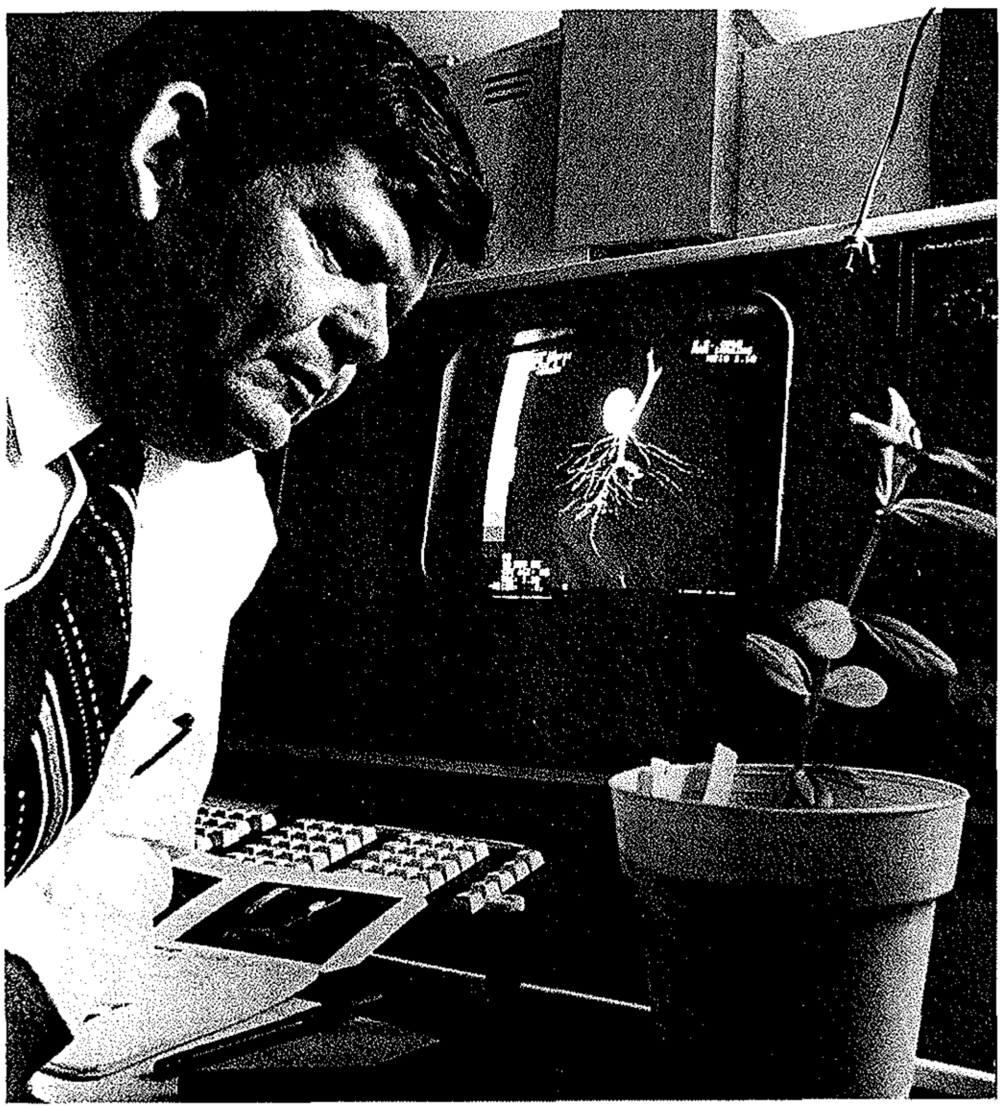

Fig. 6. H.H. Rogers at the control console of a NMR imaging machine. Image of a bean root system is shown on the display screen.

relaxation properties of protons, chiefly those associated with water, resulting in a digital image of the spatial distribution of water in plant tissue (Fig. 6). Today, intact roots less than 1 mm in diameter growing in soil or moist foam can be clearly imaged at resolutions typically less than 10 um (Cofer et al., 1989). This permits rapid production of images that simultaneously distinguish temporal changes in water distribution in root tissue, the rhizosphere and the adjacent soil at microscopic levels. All of this can be accomplished on roots growing undisturbed in the soil or in artificial substrates, something impossible until recently. Because we can calculate the proton relaxation times, the degree of matric attraction or binding of water to other substances, such as membranes, cell wall fibers, soil particles and macromolecules, may be estimated.

Many excellent references are available for NMR imaging techniques (Bottomley et al., 1984; Fukushima & Roeder, 1981; Mansfield & Morris, 1982; Roberts, 1984; Rogers & Bottomley, 1987; Witcofski et al.,1982).

SUMMARY

We have briefly described 6 techniques for studying root distribution and function in soils. The spade and core-break methods are fast and inexpensive but provide only destructive opportunities for studying roots. The mini-rhizotron technique requires the insertion of an access tube into the soil but then repeated observations can be made of roots at the soil-tube interface. The neutron radiography, computer aided tomography and nuclear magnetic resonance techniques are non-invasive but require very costly, sophisticated equipment. Each technique has a role in plant root studies. No one method is preferable for all types of plant root studies because of the compromises of time, labor, cost and accessibility of equipment.

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