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A method to separate plant roots from soil and analyze root surface area

J.G. Benjamin[1,2] & D.C. Nielsen[1]
[1]*USDA-ARS, Akron, CO 80720, Colorado, U.S.A.* [2]*Corresponding author**

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Abstract

Analysis of the effects of soil management practices on crop production requires knowledge of these effects on plant roots. Much time is required to wash plant roots from soil and separate the living plant roots from organic debris and previous years' roots. We developed a root washer that can accommodate relatively large soil samples for washing. The root washer has a rotary design and will accommodate up to 24 samples (100 mm diam. by 240 mm long) at one time. We used a flat-bed scanner to digitize an image of the roots from each sample and used a grid system with commercially-available image analysis software to analyze each sample for root surface area. Sensitivity analysis and subsequent comparisons of 'dirty' samples containing the roots and all the organic debris contained in the sample and 'clean' samples where the organic debris was manually removed from each sample showed that up to 15% of the projected image could be covered with debris without affecting accuracy and precision of root surface area measurements. Samples containing a large amount of debris may need to be partitioned into more than one scanning tray to allow accurate measurements of the root surface area. Sample processing time was reduced from 20 h, when hand separation of roots from debris was used, to about 0.5 h, when analyzing the image from an uncleaned sample. The method minimizes the need for preprocessing steps such as dying the roots to get better image contrast for image analysis. Some information, such as root length, root diameter classes and root weights, is not obtained when using this technique. Root length measurements, if needed, could be made by hand on the digital images. Root weight measurement would require sample cleaning and the advantage of less processing time per sample with this method would be lost. The significance of the tradeoff between information not obtained using this technique and the ability to process a greater number of samples with the time and personnel resources available must be determined by the individual researcher and research objectives.

Introduction

Studying soil management effects on plant root systems often is deterred by cost, both in terms of time and labor, for collecting root samples, for washing the soil from the sample and for separating the live roots from previous years' roots and other organic debris. Pit excavations are a common technique used by several researchers (Nelson and Allmaras, 1969; Allmaras and Nelson, 1971; Ehlers et al., 1983; Voorhees, 1989) to study soil management effects on root systems. This method can be very informative for examining whole root systems and deriving lateral and vertical root distributions. The method is also very destructive within the plot and does not lend itself to repeated measurements in a research plot.

Another method to study root distributions entails removing a soil core from the field, washing the soil away from the roots and measuring either root length or root area. Washing the root samples on a wire screen (Prathapar et al., 1989) or with some version of a semi-automatic elutriation system (Chotte et al., 1995; Sharma et al., 1981; Smucker et al., 1981) is common. Individual samples washed with these apparatuses can take from 3 to 10 min per sample for coarse and medium textured soils (Smucker et al., 1981) to 25 min per sample for fine textured soils (Sharma et al., 1981). Improvements on time-per-sample to wash a number

*FAX No: 970-345-2088.
E-mail: Joseph.Benjamin@ars.usda.gov

Notes

[1]Banjo Corporation, Crawfordsville, IN. Mention of specific manufacturers and trade names throughout this paper are for informational purposes only and do not imply endorsement by the USDA over similar products or manufacturers.
[2]Caplugs Incorporated, Buffalo, NY.
[3]Agfa-Gevaert N. V., Mortsel, Belgium.
[4]SPSS Inc., Chicago, IL.

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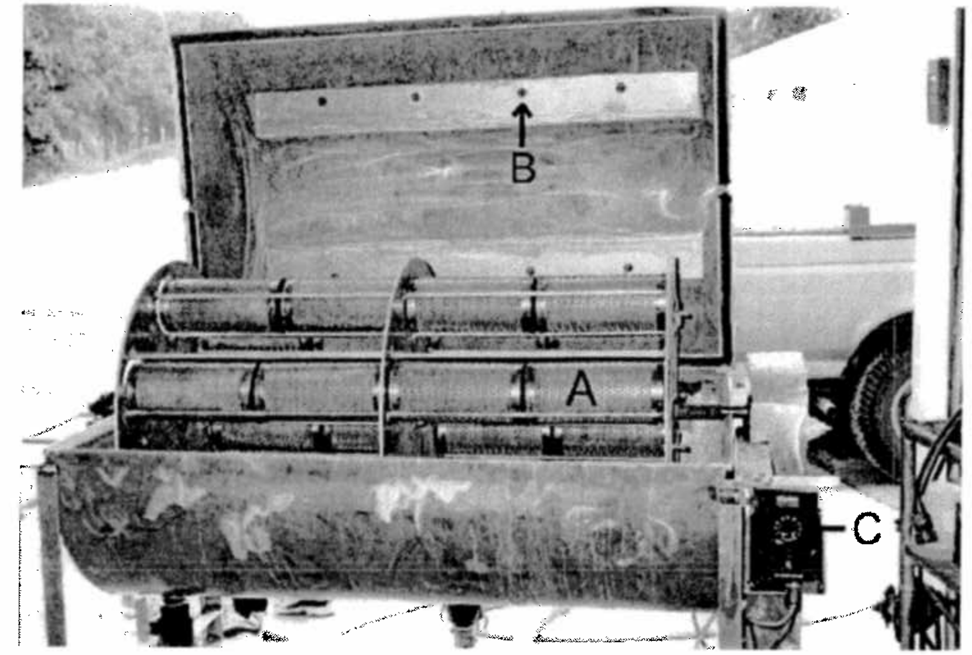

Figure 1. Photograph of root washer. Items identified in the photograph are the line strainer sample holder (A), the spray nozzles in the lid of the washer (B), and the rheostat motor control (C).

the filters containing the soil samples are dipped. As the filters emerge from the water reservoir, they are sprayed with water at 340 kPa from water nozzles (Figure 1, Item B) mounted in the lid of the washer. The washer will hold up to 24 samples for washing at one time. A motor with a drive belt turns the washer at 1.3 revolutions per minute. The rotational rate can be varied with a rheostat control (Figure 1, item C) on the motor.

The cost of construction of the washer was about $3500 US. The most expensive item was the inner rotating sample holder, which was manufactured by a local machine shop for $1500. Each line strainer cost $55. We saved money on construction costs by using a salvage aluminum barrel for the outer drum. Miscellaneous aluminum and steel bar stock, the motor, rheostat, wheels, belt and pulleys made up the rest of the costs. All construction except for the inner rotating sample holder was done by station personnel.

Root sampling and washing

Plant roots were sampled from a field of Arvika spring field pea (*Pisum sativum* L.) and from a field of chickpea (*Cicer arietinum* L.) at the Central Great Plains Research Station near Akron, Colorado. The legumes were grown on a Weld loam (fine, smectitic, mesic Aridic Paleustolls) in 2001 using no-till soil management. Samplings for roots were taken from three plants in adjacent rows approximately 170 mm apart at mid bloom growth stage for each species. A sampling tube 75 mm in diameter and 1.2 m long was used for sampling. The plant material above the soil surface was clipped level with the soil surface and removed before sampling. Any loose plant residue on the soil surface was also brushed away from the sampling site. The sampling tube was centered over the plant and a sample was taken to a 1.12-m depth. The core was sectioned into 0.225-m lengths. Each sample was placed in a plastic, sealable bag and the bags placed in a Styrofoam cooler for transport from the field. After each half day's sample collecting, the samples were placed in a refrigerator for storage until washing the next day.

Each strainer was identified by etching a pair of endcaps with a unique number. An endcap was placed on the strainer, a soil sample was removed from the plastic bag and placed in the strainer, and a second endcap was placed on the strainer. Strainers were mounted in the brackets of the washer. The water supply was connected to the washer, the lid was closed on the washer, the water was turned on, and the motor was turned on. The washer was allowed to operate until the effluent from the washer contained very little soil. Sample wash time was generally 1.5 h for the Weld

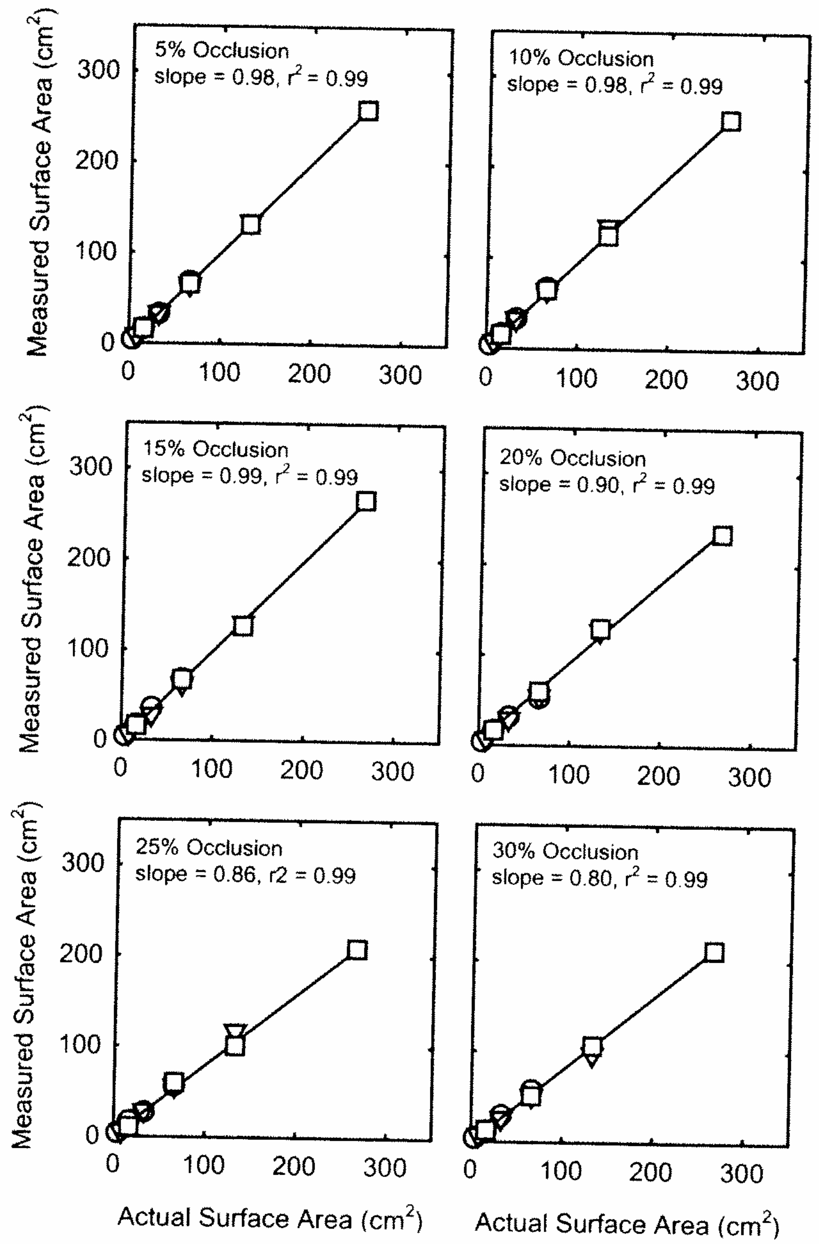

Figure 4. Effect of percentage occluded area on accuracy and precision of projected area measurements of copper wire. The circles indicate 0.5 mm diam. wire, the triangles indicate 1.0 mm diam. wire, and the squares indicate 2.0 mm diam. wire. Grid size for measurements was 2.1 mm.

materials in the sample making the determination of a positive intersection with grid difficult. Including samples with > 15% debris coverage in the regression of clean vs. dirty root area measurement decreased the slope to 0.80 and decreased the r^2 to 0.82.

Discussion

The root washer worked well to easily and quickly separate plant roots and other organic materials from the soil. Up to 24 samples could be washed in about 1.5 h, allowing samples to be quickly processed after sampling and minimizing deterioration of the root materials. The root washer would be less effective in

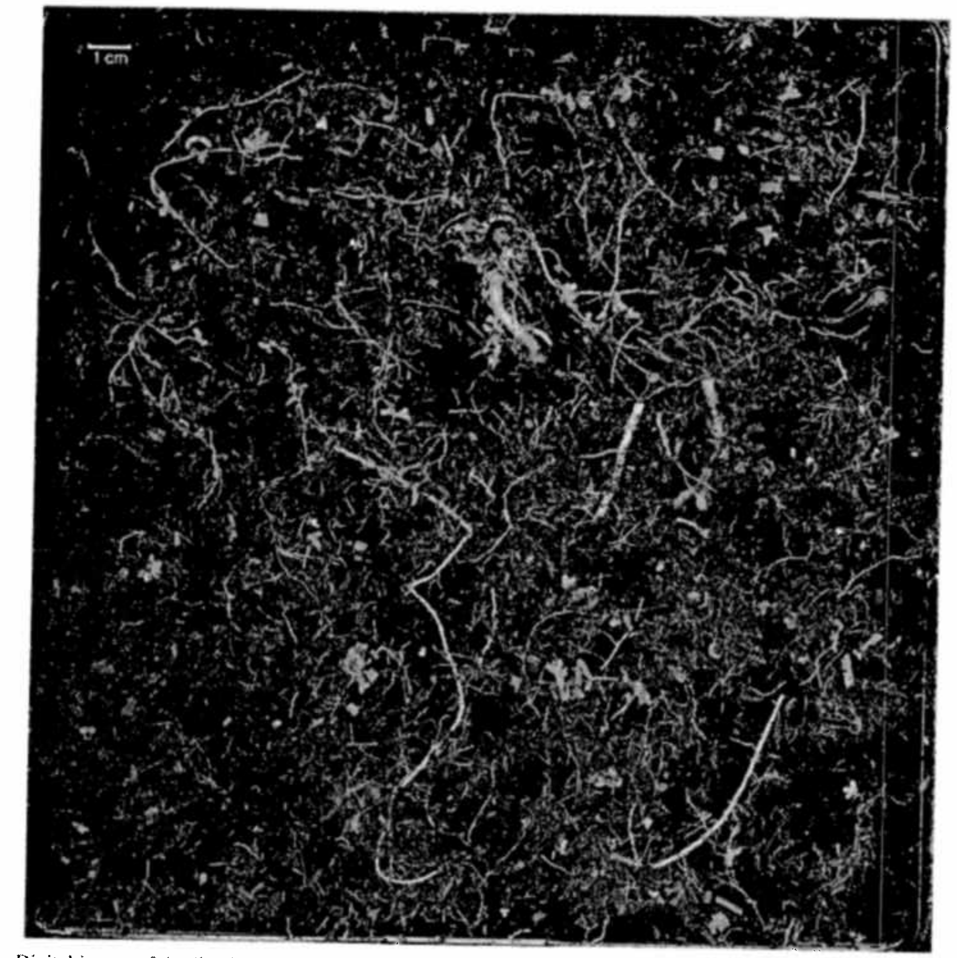

Figure 2a. Digital image of Arvika field pea root and debris, 0 to 23 cm depth before cleaning. 38% of total area covered by debris.

distinguish the presence of a root in the observation field, while samples with a small amount of debris had little effect on the measurement of the sample. A systematic analysis was conducted to determine the amount of the viewing area that could be covered by debris before interfering with measurement accuracy. Known amounts of 'debris' consisting of 5.5 mm diameter paper punches from black construction paper were glued to sample trays with a spray adhesive. Each punch had an area of 23.75 mm^2. A sufficient number of punches was placed on the 230 mm square clear plastic tray to obtain 5%, 10%, 15%, 20%, 25% and 30% occlusion of the viewing area. Individual punches were allowed to touch, forming strings of occlusion on the image, but the punches were not allowed to overlap each other. The same copper wire was used as in the calibration procedure explained above. Samples consisting of 0.25, 1.0, 2.0, and 4.0 m lengths of copper wire were laid on top of the 'debris' and scanned.

The scanning procedure and area analysis was conducted in the same fashion as for determining optimum grid size using a grid density of 2.1 mm (100 × 100 intersections).

Results

Precision and accuracy of the copper wire surface area measurements increased with increasing grid density (Figure 3). Precision of the technique was indicated by the r^2 of the regression between the measured and actual surface area of the wire samples. The greatest improvement in precision, as indicated by an increased r^2 from 0.89 to 0.98, occurred when decreasing grid size from the 14.4 mm grid spacing with 225 possible intersections to the 8.6 mm grid spacing with 625 possible intersections. All other grid sizes had approximately the same r^2 as the 8.6 mm grid, with the r^2