



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

J.G. Benjamin^{1,2} & D.C. Nielsen¹

¹USDA-ARS, Akron, CO 80720, Colorado, U.S.A. ²Corresponding 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 dyeing 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

of samples can be had by operating several elutriators at once.

After separating the roots from soil, a method is needed to quantify the amount of roots in the sample. The line intersect method of Newman (1966) or a variation thereof (Reicosky et al., 1970; Tennant, 1975) has been used to quantify root length density. Recent advances in digital image analysis have led to the development of automated techniques to measure root length and surface area. Lebowitz (1988) and Zoon and van Tienderen (1990) used a video camera to digitize root images for measurement of root length. The images were manually converted to binary images of black and white pixels and the length of root segments were analyzed. Kaspar and Ewing (1997) developed a computer program to automatically convert a root image obtained from a desktop scanner to a binary image suitable for analysis. Farrel et al. (1993) showed that comparable results could be had with either manual or digital measurement of root lengths using the line intersect method. Dowdy et al. (1995) and Kimura and Yamasaki (2001) found that easily-obtained public domain image analysis software could be used to quantify the length and diameter of root segments. Kokko et al. (1993) used a slightly different approach to quantify roots from a soil sample by counting the number of pixels projected on a 2-dimensional image to determine root surface area. Costa et al. (2000) developed methodology to subsample large root systems so that not all the root material is needed for root length measurements by image analysis.

These methods (Costa et al., 2000; Dowdy et al., 1995; Farrel et al., 1993; Kaspar and Ewing, 1997; Kimura and Yamasaki, 2001; Kokko et al., 1993; Pan and Bolton, 1991) often require that the roots be dyed to a uniform color to increase the contrast between the sample and background. Dying the roots and other soil debris makes it difficult to distinguish between the two. Another requirement of these methods is the need for the separation of living roots and previous years' roots and other organic debris. Manually separating roots and debris is both time consuming and tedious. Dowdy et al. (1998) developed algorithms for the automatic separation of roots from soil debris, using public domain image software, based on length:width ratios of the projected items. Their technique requires separation between each root and each item of debris to allow the computer to delineate each item and accurately distinguish between the two.

Even with recent advances in image analysis techniques, the human eye and brain remain the best and

most accurate method to distinguish the subtle differences between living roots and soil organic debris. The automatic gray-scale imaging of Kaspar and Ewing (1997) relies on the human brain to make the preliminary distinctions between root and debris. Some of the criteria to separate living roots from previous roots and soil debris include color, dimensions, and texture. A combination of all three criteria is needed to distinguish roots from debris. Patena and Ingram (2000) and Ingram and Leers (2001) had good success for measuring root lengths on minirhizotron images by tracing the root image by hand on a computer screen with a mouse pointer.

One objective of this study was to design and build a root washing mechanism that allows the rapid removal of soil away from the roots from multiple samples. Another objective was to develop a method to quantify the root surface area of roots washed from a soil sample and quantify a threshold of root debris in a sample that interferes with the accurate measurement of root area.

Materials and methods

Root washer

The original concept for the rotary root washer came from a weed seed washer designed and built by Wiles et al. (1995), which used line strainers housed in a rotary drum to contain the samples for washing. Our perceived disadvantage of their sampler was the small sample size (65 mm diam. by 175 mm long) that the washer could contain. We designed this washer to accommodate larger samples. The root washer was designed around a commercially-available line strainer for sprayer systems (Banjo LS-350¹). The filter body (Figure 1, Item A) is 100 mm diameter and 240 mm long. The filter consists of a stainless steel screen cylinder with 300 μm openings housed within a stainless steel cylinder with 6.25 mm openings. End caps (Caplugs² model BPF-4) were obtained that gave a force fit on the ends of the filter. The seam between the end reinforcing ring and the interior screen was filled with silicone cement to prevent roots being trapped in this area. The washer itself was constructed from aluminum. A 30 mm wide slot spanning the entire rear of the tank was cut 0.25 m above the bottom of the tank for removal of mud slurry resulting from washing the soil from the roots in the filters. This creates a reservoir of water in the lower section of the washer into which

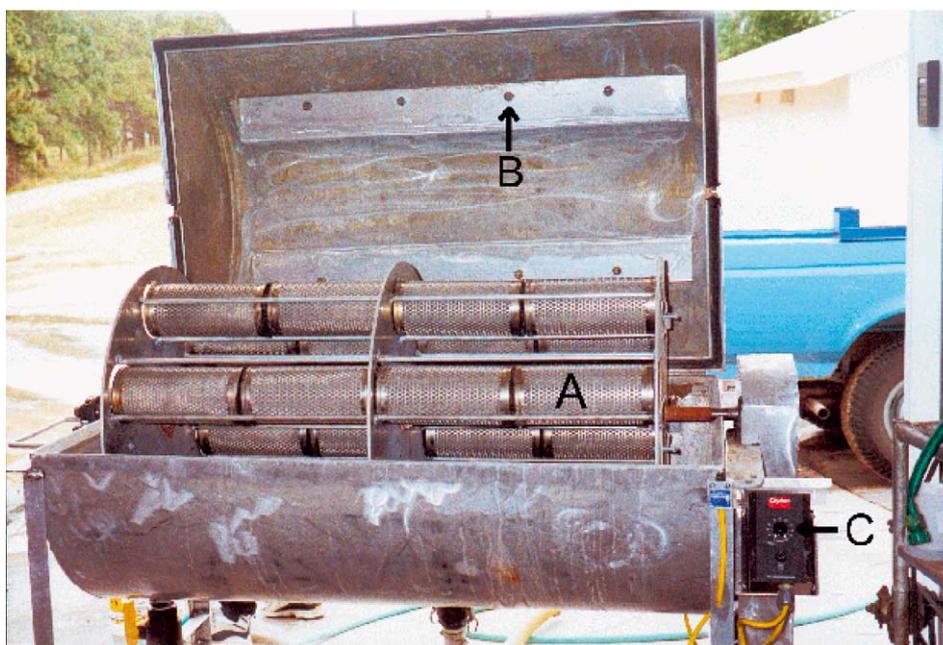


Figure 1. Photograph of root washer. Items identified in the photograph are the line strainer sample holder (A), the spray nozzles in the lid of the washer (B), and the rheostat motor control (C).

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 man-

agement. 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

loam soil. After washing, the motor was stopped, the water was turned off, and the filters were removed one at a time. Roots and any plant residues and sand too large to pass through the screen were washed onto a 300 μm screen. The material on the screen was removed with Teflon tweezers and placed in a plastic sample cup. The sample was covered with methanol and placed in refrigerated storage until time for sample analysis. All transfers were made with the help of a 3 \times lighted magnifier to help ensure complete transfer of root materials.

Root surface area analysis

Before cleaning, each sample was spread on a 230 \times 230 mm clear plastic tray. The tray was filled with methanol to a level above the thickest root to minimize images with many menisci between the surface of the root and the surface of the fluid. Care was taken to spread the sample uniformly across the entire surface of the tray. The tray was covered with a clear plastic lid and then covered with a black cloth to achieve a dark background for scanning (Figure 2a). Scans were made with an Agfa³ SnapscanTM E40 flatbed scanner at 118 pixels per cm (300 dpi). After cleaning an additional scan was made (Figure 2b).

Roots and debris were separated manually by placing the sample in a tray and examining the sample with the aid of a 3 \times lighted magnifier. Debris and roots were picked into separate containers using Teflon tweezers. Root samples from the surface layer of soil had many roots and the cleaning procedure was confounded with the large amount of residue from previous crops. Cleaning samples from the 0 to 225-mm layer, with the largest amount of debris, often took more than 20 h. Root samples from deeper in the soil profile had fewer roots and much less debris than the surface samples and could be cleaned in about 1/2 h or less.

We used a grid overlay technique similar to that of Kokko et al. (1993) to convert the area of a projected image from a 2-dimensional scan to 3-D root surface area. To test the precision and accuracy of the technique we prepared samples of known lengths of 0.5 mm (24 ga.), 1.0 mm (18 ga.), and 2.0 mm (12 ga.) copper wire. Lengths of 0.25, 0.5, 1.0, 2.0, 3.0, and 4.0 m of each diameter wire were cut into arbitrary sections between 1 and 6 cm long. The surface area of the wire sample (A_w) was calculated by

$$A_w = \pi D_w L_w \quad (1)$$

where D_w is the diameter of the wire and L_w is the total sample length.

The wires for each diameter and length combination were spread across a 230 \times 230 mm clear plastic tray and the sample was scanned with an Agfa SnapscanTM E40 flatbed scanner at 118 pixels per cm (300 dpi). Each scan was loaded into SPSS, Inc.⁴ Sigma-ScanTM image analysis software to determine surface area. A series of grids with 14.4, 8.6, 6.5, 4.3, 2.9 and 2.1 mm line spacing (corresponding to 15 \times 15, 25 \times 25, 33 \times 33, 50 \times 50, 75 \times 75, and 100 \times 100 grid densities, respectively, on the computer screen) were placed over the image. A grid was randomly placed on the image and the positive intersections were hand counted. Triplicate grids and counts were done for each grid density. The number of intersections that crossed the wire (I_w) was counted and compared with the total intersections possible (I_t). The projected area of wire (A_p) was calculated by

$$A_p = A_t I_w / I_t, \quad (2)$$

where A_t is the total image area. The surface area of the wire measured from the image analysis (A_s) was then calculated as

$$A_s = \pi A_p. \quad (3)$$

To calculate the surface area of the roots contained in the soil samples each digital root image was loaded into the SigmaScanTM program and a grid with approximately 2 mm line spacing was superimposed over the image. The number of positive intersections of a grid point with a root (I_r) and the number of positive intersections with debris (I_d) for the uncleaned samples were counted by the operator. The total number of intersections (I_t) was recorded. The projected area of roots (A_r) was calculated by

$$A_r = A_t I_r / I_t. \quad (4)$$

The projected area of the debris was calculated by

$$A_d = A_t I_d / I_t. \quad (5)$$

The surface area of the roots (A_{sa}) calculated from the projected area was

$$A_{sa} = \pi A_r. \quad (6)$$

Very dirty, high-root-density, samples required about 30 min per sample for analysis. Less dirty, low-root-density, samples or samples that had been cleaned required approximately 10 min per sample for analysis.

It was observed that samples with a large amount of debris interfered with the ability of the operator to

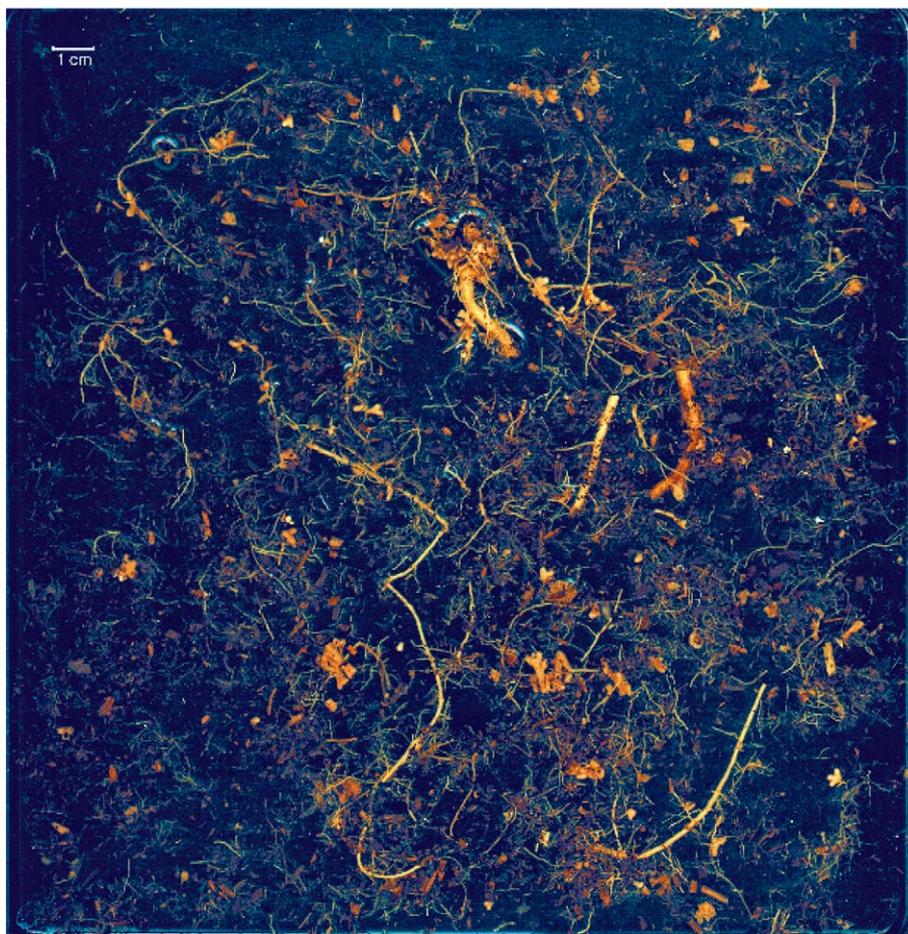


Figure 2a. Digital image of Arvika field pea root and debris, 0 to 23 cm depth before cleaning. 38% of total area covered by debris.

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 mm². 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

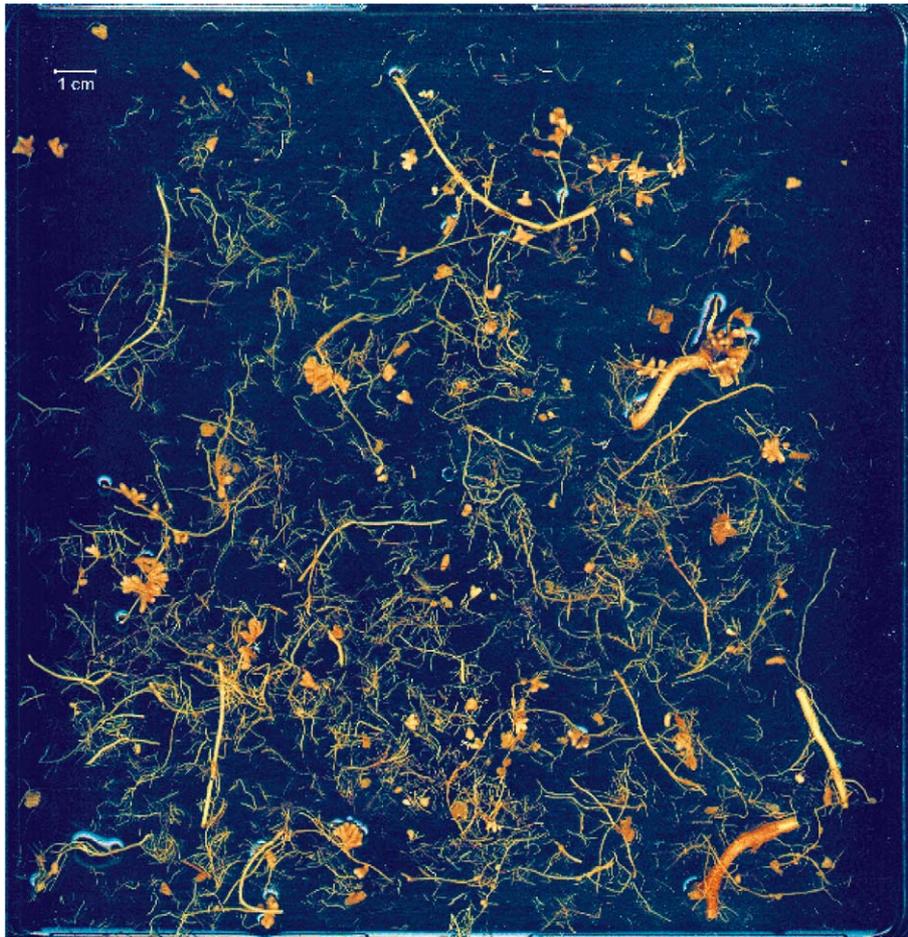


Figure 2b. Digital image of Arvika field pea root, 0 to 23 cm depth, after cleaning.

greater than 0.98. The accuracy of the technique was indicated by the slope of the regression line. A slope of 1.0 would indicate exact agreement between the measured and actual values. Most regression slopes were between 0.78 and 0.87, indicating an underestimation of the true surface area of the samples. The grid density of 2.1 mm (100×100 intersections) gave the greatest precision and accuracy with a slope of 0.99 and an r^2 of 0.99. There did not appear to be a bias due to wire diameter for precision or accuracy. The slope and r^2 of the regression of measured vs. true surface area for each separate wire diameter was similar to that of the combined regression.

Accuracy of the wire area measurements decreased as the amount of debris occluding the image increased (Figure 4). Images with 5%, 10%, and 15% of the image area covered with debris had similar accuracy as for images of no debris, with a slope of the regression of measured vs. true projected area being almost

1 and an r^2 of 0.99. As the debris increased over 15%, it was more difficult to determine whether a wire lay under an intersection, therefore fewer sample counts were made and accuracy, as measured by the slope of the regression of measured vs. actual wire area, was decreased. Precision, however, remained high, with an r^2 of over 0.99.

Root area measurements for cleaned and uncleaned samples (Figure 5) were similar except for surface samples with high root density that also contained more than 15% of the image area covered by debris. There was not a difference between plant species. A linear regression of the clean vs. dirty samples for all samples containing < 15% debris coverage had a slope of 0.97 and an r^2 of 0.96. For samples that had > 15% of the image area covered with debris, there was an underestimation of the root surface area when measured on the dirty sample. This is likely due to occlusion of the roots in the image by other organic

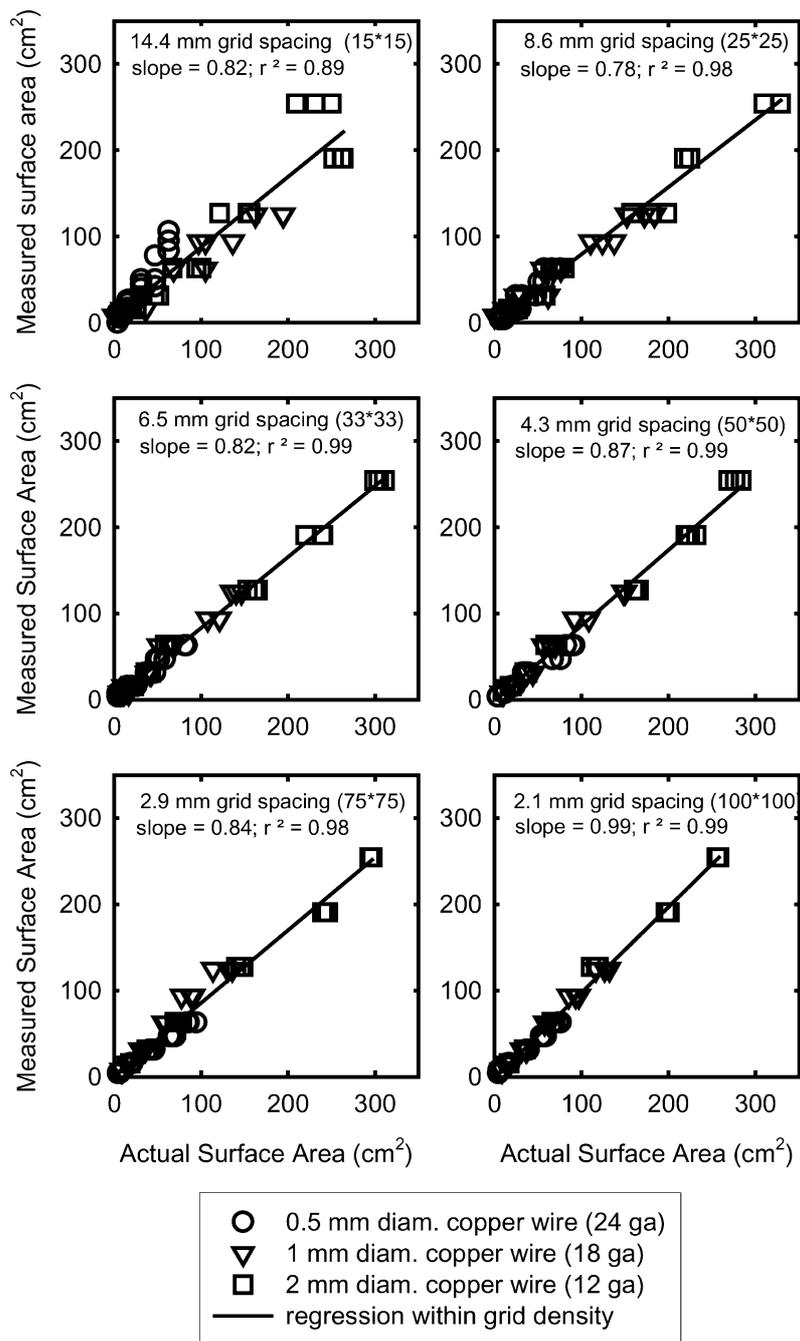


Figure 3. Results of surface area calculations of known lengths and diameters of copper wire using the grid overlay technique with SigmaScan™ image analysis software.

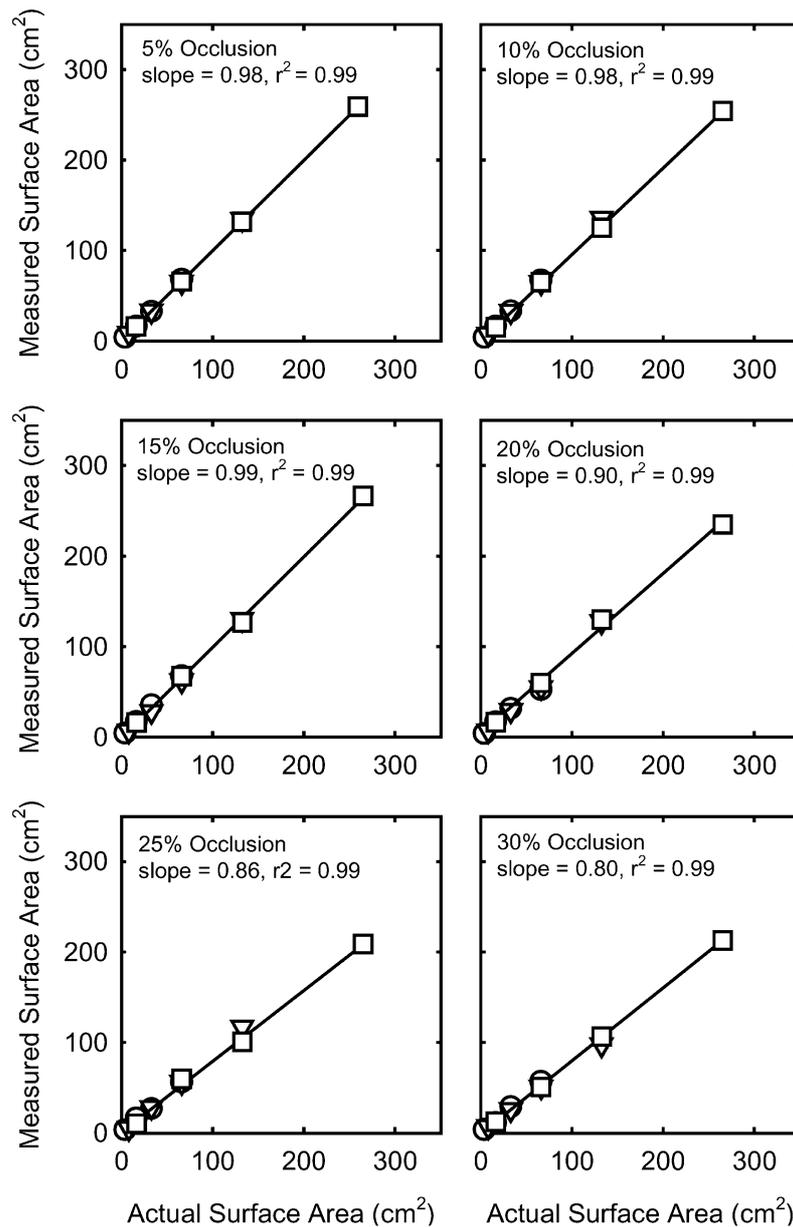


Figure 4. Effect of percentage occluded area on accuracy and precision of projected area measurements of copper wire. The circles indicate 0.5 mm diam. wire, the triangles indicate 1.0 mm diam. wire, and the squares indicate 2.0 mm diam. wire. Grid size for measurements was 2.1 mm.

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

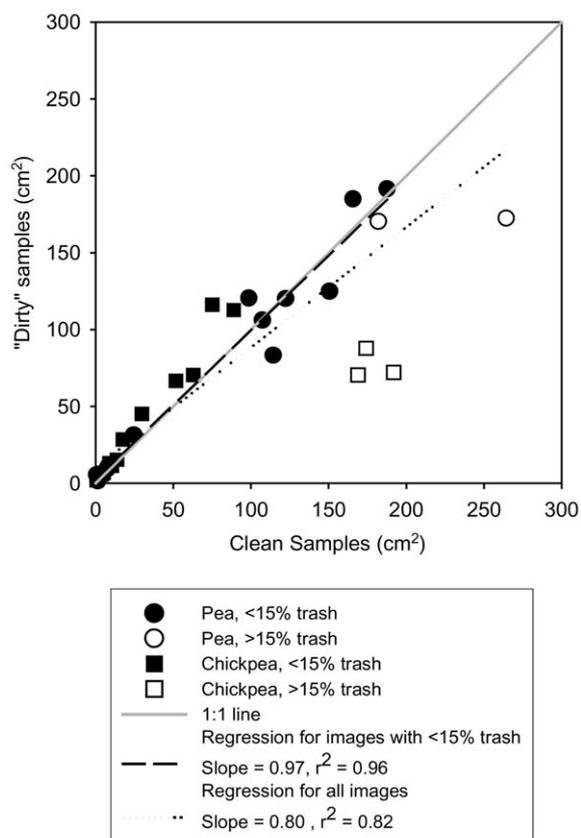


Figure 5. Comparison of total root surface area of 'dirty' vs. cleaned samples of pea (*Pisum sativum* L.) roots and chickpea (*Cicer arietinum* L.) roots. The circles indicate root area of the pea and the squares indicate the root area of the chickpea. The open symbols indicate samples where over 15% of the projected area was non-root trash. Grid size for measurements was 2.1 mm.

sandy soils because the sand would be held in the sample container with the roots. A separation technique such as letting the sand settle in a container and skimming the roots and organic debris off the surface may be needed to remove the sand from the sample.

Surface area measurements of standards were accurate when a small enough grid size was used. For this procedure, a grid density of 2 mm or less between lines was the most accurate, with a slope of the correlation line nearly 1 and an r^2 of 0.99. The precision of measurements using coarser grids was still good, but accuracy suffered and the surface area of the sample was underestimated.

A relatively small amount of debris in the field of view had little effect on measurement precision or accuracy for determining root surface area. Larger amounts of debris in the field of view, in this case over 15% of the image occluded by debris, caused interference with the ability of the operator to determine the existence of roots in the field of view and whether a grid intersection coincided with the root. Partitioning the sample into more than one scanning tray may be necessary to allow accurate measurements of the root materials in very dirty samples. If root data such as root weights are needed, sample cleaning would still be necessary. 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 dyeing the roots to get better image contrast or separation of the sample into many scans to get complete separation of roots and debris.

We recognize that some information, such as root length, root diameter classes and root weights, is not obtained when using this technique. Information on total root length is not determined directly but could be measured using the tracing techniques of Patena and Ingram (2000) and Ingram and Leers (2001). If root weights were needed, sample cleaning would still be necessary and the advantage of less processing time per sample with this method would be lost. It must be determined by the individual researcher, based on the research objectives, whether the information not obtained using this technique is counterbalanced by the ability to process a greater number of samples with the time and personnel resources available.

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Notes

¹Banjo 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.

²Caplugs Incorporated, Buffalo, NY.

³Agfa-Gevaert N. V., Mortsel, Belgium.

⁴SPSS Inc., Chicago, IL.

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