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Elimination of non-root residue by computer image analysis of very fine roots

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Abstract

Hydropneumatic root separation from field soil collects organic non-root residues. This study compares manual cleaning to electronic cleaning with image analysis of carrot roots from field experiments. Washed and stained very fine roots of carrots (diameter 0.15 mm) were video-recorded by using a high-resolution robotic camera system. Fibrous root lengths and widths of roots from samples grown in fine sand ($n = 160$) and organic soils ($n = 150$) were determined by automated image processing of video-recorded images. During video-recording, some non-root residue remained with the extracted fine roots, especially in samples from organic soils, where residue materials comprised partially decomposed straw and peat. In computerized image analysis, materials with length-to-width ratios of ≤3:1 were deemed non-root debris and discarded electronically. The 3:1 length-to-width ratio was chosen for distinguishing long thin root images from short organic residues. Results show that more non-root residue was recovered by image analysis than was recorded by dry weight estimation after manual cleaning. Length, surface area and volume averaged 15%, 18% and 24% of non-root residue in the manually fully cleaned samples (residue estimation 0%, i.e. no visible debris recorded visually) of fine sand soils ($n = 80$). Length, surface area, and volume averaged 20%, 25% and 33% ($r = 0.55$, $p < 0.0001$), respectively, of non-root residue in the organic soil samples with residues <10% (g residues/g roots and residues) ($n = 71$). Both root and residue densities were higher at soil depths of 0–25 cm than in deeper subsoils, where the percentages of discarded residue were highest. Electronic non-root separation effectively eliminated short and partially decomposed organic material that was hardly visible or otherwise difficult and time-consuming to remove manually.

Keywords: Computer image analyses; Non-root residue; Root length; Root diameter; Digital files; Website root image analyses; Quantification of root morphology

1. Introduction

Modern image analysis methods for comprehensive examination of individual root lengths and diameter are needed to improve our evaluations of water and nutrient uptake efficiencies by root systems (Nielsen, 1979; Kirkham et al., 1998). Fine root diameters of less than 0.5 mm are critical for accurately defining morphological functions of root systems. In addition, root data have taken on renewed significance, with interest currently being focused on C sequestration and soil CO_2 fluxes (Campbell and de Jong, 2001). Automated root intersect counting by video camera imaging (Voorhees et

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al., 1980) has been extended to root image analyses (length, width, branching) by using a scanner and a microcomputer (Zoon, 1990; Pan and Bolton, 1991; Ewing and Kaspar, 1995; Zobel, 2003). For measuring projected root area or root surface area, image analysis procedures have been developed for clean, washed roots (Harris and Campbell, 1991; Kokko et al., 1993), and modified for washed root samples from fields containing residues (Smucker, 1993; Dowdy et al., 1998). This modification is of great importance since non-root residue can be a considerable source of error in estimates of root data obtained with image analysis. This study provides comparisons of root and non-root lengths, surface areas and volumes by image analysis of very fine roots with none or some non-root residues.

2. Materials and methods

Destructive root sampling of the carrot (*Daucus carota* L.) was performed from a fine sand (Aquic Haplocryoll) (Yli-Halla and Mokma, 2001) and an organic (Aquic Cryoboroll) soil in Finland, as described by Pietola and Smucker (1998). Roots and associated residue were washed from soils using a hydropneumatic elutriator (Smucker et al., 1982). The elutriation method separated all organic materials which were less dense than the mineral fraction of the soil, retaining up to 99.4% of all roots >0.05 mm. However, from the organic soil, considerable quantities of organic debris were also extracted along with the fine roots. Before imaging, root samples were manually cleaned, but some residual debris remained, especially in organic soil samples.

Following the video imaging procedure described by Pietola and Smucker (1998), root lengths and widths, including some residual non-root material, were determined by automated image processing of root images (Smucker, 1993) in the Root Image Processing Laboratory at Michigan State University. The website http://www.rootimage.msu.edu accesses the necessary software for quantification of root morphology and removing non-root materials.

Following imaging, the dry weight of root samples ($DW_{weighed}$) was determined after oven drying for 48 h at 70 °C. After manual cleaning, dry weights of non-root material in the root samples (DW_{tray}) was estimated as follows:

$$DW_{tray} = E \times DW_{weighed} \tag{1}$$

where E is the visually estimated percentage of the non-root materials remaining in the tray of the removed, weighed portion of non-root material. Volume-based visual estimation of all remaining non-roots was performed by the same person. Samples that could not be cleaned to $E = 0\%$, included some short, partially decomposed organic material left mostly at the bottom of the tray, below a water film of 3 mm, thus having a density (σ) of over 1 g cm^{-3}. The non-root material included larger straw pieces floating on the water surface ($\sigma < 1$ g cm^{-3}) as well as more decomposed material below the water surface ($\sigma > 1$ g cm^{-3}). Estimation of the average density of non-root debris was thus 1 g cm^{-3}, close to that of carrot root material (Pietola and Smucker, 1998).

Because of excessive roots, some samples were divided into two to four subsamples to avoid overlapping of roots in the trays during imaging. Data of split samples were combined before statistical analyses ($n = 310$). Sample trays of 18.5 cm × 19.5 cm were image-recorded with a 12.5 mm C-mount lens, coupled with a 4× C-mount extender and a 10 mm C-mount extension tube (Vision Components Inc., Burmingham, MI, USA) for precise determination of root diameters >0.15 mm.

Images of stained root samples were analyzed by the PC-based microcomputer image analysis system (Smucker et al., 1987; Smucker, 1990, 1993). A binary image was created from the original grayscale image. Measurements of root total length and width classes were determined. Average root width classes were 0.15, 0.40, 0.75, 1.25 and 1.95 mm with a root image resolution of 276 pixels cm^{-1} in the Michigan State University (MSU) Root Image Processing Laboratory (RIPL) system. The processing time for each image was 1.5–2 min, depending on the quantity of non-root residues in the image. Eighteen morphological components of carrot roots were quantified by this robotic camera computer image processing approach. Lengths, surface areas and volumes of the five root-width classes provided 15 distinctly different root morphologies for each root system. Sums of these five root width classes provided three additional root parameters for each root sample. Ratios between these values for the different root widths could also provide more root information, relating morphology to specific soil components or plant variety. Total root length at a site represented the sum of the lengths of all layers and width classes. Root surface area (SA) was calculated using Eq. (2), which is based on the assumption that carrot roots are cylindrical:

$$SA = 2\pi r L \tag{2}$$

Table 1
Percentages of non-root debris (S.D.) and [minimum–maximum] in all root and debris material, on the basis of length, surface area and volume of roots and non-root residues in manually fully cleaned (all residues removed to 0% (g residues/g roots and residues)) in fine sand samples ($n = 80$) and in partly cleaned (<10% (g residues/g roots and residues)) organic soil samples ($n = 71$)

	Fine sand soil	Organic soil
Length[a] ($m\,m^{-1}$)	14.7 (4.4) [7.4–31.9]	20.2 (5.0) [10.4–38.8]
Surface area[b] ($m^2\,m^{-2}$)	18.3 (5.6) [9.2–47.2]	24.9 (5.7) [12.7–45.1]
Volume[c] ($m^3\,m^{-3}$)	23.8 (7.4) [10.4–57.3]	32.5 (6.9) [17.9–52.9]

[a] The ratio of the length of non-root residues to the total length of roots and non-root residues × 100.
[b] The ratio of the surface area of non-root residues to the total surface area of roots and non-root residues × 100.
[c] The ratio of the volume of non-root residues to the total volume of roots and non-root residues × 100.

Root volume (V) was calculated using Eq. (3), where r is the radius and L is the length of root or non-root materials:

$$V = \pi r^2 L \tag{3}$$

Materials with lengths that were ≤3 times their widths were identified as non-root debris and discarded electronically by the RIPL computer image software, within each width class. The 3:1 length-to-width ratio was chosen for distinguishing long thin root images from short organic residues. Relationships between root and non-root parameters were determined by linear correlation and regression analyses. For correlation and regression analyses, only clean or partially cleaned samples were used, i.e. 80 samples from sandy soil and 71 samples from organic soil. In fine sand, all samples ($n = 160$) consisted of, in addition to the roots, mainly small short non-root objects, averaging 2% (g/g) based on the estimation yielded by using Eq. (1). Half ($n = 80$) of the samples were manually totally cleaned, with no visible debris left, i.e. $E = 0\%$ (g/g). No samples from organic soil ($n = 150$) were fully manually cleaned ($E = 0\%$, g/g). However, only small amounts of debris (<10% (g/g), Eq. (1)) remained in 71 organic soil samples. These partially cleaned organic soil samples were used for correlation and regression analyses.

3. Results and discussion

Despite manual removal of all residues ($E = 0\%$), image processing of fine sand samples ($n = 80$) found 15% non-root residue on a length basis, and 24% non-root residues on a volume basis (Table 1). Identification and removal of non-root residues from organic soil root samples averaged 20% on a length basis, and 33% on a volume basis, while the amount of non-root residue on a dry weight basis was estimated at <10% (g residues/g roots and residues) ($n = 71$). Thus, a greater percentage of non-root residues were identified by image analyses than by dry weight estimations. These values demonstrate that a 3:1 length-to-width ratio was sufficient to separate non-root debris of both organic and mineral origin from very fine roots. According to Dowdy et al. (1998), a 15:1 object length-to-width ratio was the best threshold to separate root images from organic residues associated with corn cropping systems containing larger root diameters.

In fine sand, most carrot roots were in the width class of 0.15 mm, representing an average length of 10.3 m; the total length of all width classes was 11.7 m (Table 2). By contrast, the average non-root length of all fine sand root samples ($n = 160$) was 1.4 m. Most residue (71%) recorded by image processing separation was very thin, belonging to the width class of 0.15 mm, and was neither identified nor removable by hand picking. Very few larger residue objects in width classes ≥0.75 mm (which are visible, yet missed during routine manual removal) were recorded by the computer.

In organic soil, 63% of non-root residue lengths ($n = 150$) consisted of thin objects (width class 0.15 mm) and 11% represented wider objects (≥0.75 mm) (Table 3). Almost none of the root material was recorded in the width classes of 1.25 and 1.95 mm. The lack of large roots is in accordance with visual observations that carrot roots were seldom wider than 1.5 mm and confirms that wide straw-like materials with length-to-width ratios below 3 were discarded by the computer algorithms as non-root residues.

The correlation coefficients between computer-measured length, surface area and volume of non-root materials and dry weights of non-root materials recorded from organic soil samples with <10% (g/g) non-root materials ($n = 71$) averaged $r = 0.56$ ($p < 0.0001$) within all width classes (Table 4). Within each width class, correlation coefficients were similar for length, surface area and volume, because root radii in Eqs. (2) and (3) were constant. The closest correlation

Table 2
Average length, surface area and volume of roots and non-root residues in all fine sand root samples ($n = 160$) across five width classes, averaging 2% non-roots (g residues/g roots and residues) based on the estimation yielded by using Eq. (1)

	Width class (mm)					
	0.15	0.40	0.75	1.25	1.95	Sum
Roots						
Length (m)	10.32	1.293	0.076	0.003	0	11.70
Surface area (cm^2)	48.67	16.25	1.795	0.135	0.007	66.85
Volume (cm^3)	0.183	0.162	0.034	0.004	0	0.387
Non-root residues						
Length (m)	1.025	0.327	0.077	0.005	0	1.434
Surface area (cm^2)	4.832	4.110	1.806	0.178	0.059	10.99
Volume (cm^3)	0.018	0.041	0.034	0.006	0.003	0.102

Table 3
Average length, surface area and volume of roots and non-root residues in all organic soil root samples ($n = 150$) across five width classes, averaging 15% non-roots (g residues/g roots and residues) based on the estimation yielded by using Eq. (1)

	Width class (mm)					
	0.15	0.40	0.75	1.25	1.95	Sum
Roots						
Length (m)	13.95	2.940	0.342	0.010	0	17.24
Surface area (cm^2)	65.73	36.95	8.056	0.399	0.013	111.14
Volume (cm^3)	0.246	0.369	0.151	0.012	0.001	0.792
Non-root residues						
Length (m)	3.464	1.403	0.556	0.051	0.004	5.479
Surface area (cm^2)	16.32	17.63	13.11	2.015	0.272	49.347
Volume (cm^3)	0.061	0.176	0.246	0.063	0.013	0.559

($r = 0.60$, $p < 0.0001$) between non-roots separated by image processing and the estimated dry weights of these materials were found in the width class of 1.25 mm, indicating that objects of this size were efficiently removed.

The regression equation ($p < 0.001$) for non-root debris lengths (y, m) for organic soil materials with <10% (g/g) was as follows:

$$y = \underset{(0.32)}{2.67} + \underset{(0.08)}{0.43}x \quad (R^2 = 0.31) \tag{4}$$

where x is non-root debris dry weight (mg) for 71 samples. Standard deviations of the coefficients are in parentheses below the equation. The regression equation ($p < 0.0001$) for non-root debris surface area (y, cm^2) was as follows:

$$y = \underset{(2.78)}{21.1} + \underset{(0.67)}{3.85}x \quad (R^2 = 0.32) \tag{5}$$

where x is non-root debris dry weight (mg).

Table 4
Correlation coefficients between length, surface area or volume of non-root residues and non-root dry weights extracted from organic soil samples ($n = 71$) of <10% debris (g residues/g roots and residues), in five width classes separately, and in all width classes combined

Width class (mm)					
0.15	0.40	0.75	1.25	1.95	All
0.30*	0.38**	0.43**	0.60***	0.50***	Length: 0.56***; surface area: 0.57***; volume: 0.53***

* $p < 0.01$.
** $p < 0.001$.
*** $p < 0.0001$.

Table 5
Lengths and surface areas of roots and non-root residues per soil volume in different soil horizons (with S.D.) for all samples of fine sand and organic soil originated from different soil depths ($n = 31$ for each layer)

Soil depth[a]	RLD[b] ($cm\,cm^{-3}$)	DLD[c] ($cm\,cm^{-3}$)	RSAD[d] ($cm^2\,cm^{-3}$)	DSAD[e] ($cm^2\,cm^{-3}$)
0–5	6.49 (1.70)	0.96 (0.47)	0.36 (0.08)	0.07 (0.04)
5–10	5.34 (1.46)	0.99 (0.67)	0.31 (0.09)	0.08 (0.06)
10–15	4.87 (1.29)	1.05 (0.65)	0.30 (0.09)	0.09 (0.07)
15–20	4.93 (1.43)	1.17 (0.87)	0.30 (0.10)	0.10 (0.09)
20–25	4.31 (1.38)	1.10 (0.69)	0.27 (0.09)	0.10 (0.07)
25–30	3.01 (1.78)	0.84 (0.64)	0.19 (0.11)	0.07 (0.05)
30–35	1.89 (1.41)	0.73 (0.83)	0.13 (0.10)	0.07 (0.08)
35–40	1.82 (1.41)	0.53 (0.46)	0.12 (0.09)	0.05 (0.04)
40–45	1.70 (1.18)	0.52 (0.45)	0.11 (0.08)	0.05 (0.04)
45–50	1.57 (1.30)	0.44 (0.45)	0.11 (0.09)	0.04 (0.04)

[a] Volume of each soil layer 3534 cm^3.
[b] RLD (root length density) = length of roots:soil volume.
[c] DLD (debris length density) = length of non-root residues:soil volume.
[d] RSAD (root surface area density) = surface area of roots:soil volume.
[e] DSAD (debris surface area density) = surface area of non-root residues:soil volume.

The regression equation ($p < 0.0001$) for non-root debris volumes (y, cm^3) was as follows:

$$y = \underset{(0.03)}{0.20} + \underset{(7.92)}{41.4}x \quad (R^2 = 0.28) \tag{6}$$

where x is non-root dry weight (g). These results show that image analysis discarded a considerable portion of the residual non-root debris in root samples that had been manually cleaned.

Both fine textured and organic soils had more roots and residue in the plow layer than in the subsoil (Table 5). Significantly greater root length densities were found at a soil depth of 0–30 cm, significantly higher debris length densities at 10–35 cm and significantly greater debris surface area densities at 15–25 cm. Significantly smaller root length densities, root surface area densities and debris surface area densities were recorded at soil depths of 30–50 cm.

The percentages of non-root materials averaged 23% of the total lengths measured at soil depths of 25–50 cm, but the percentage was lower (17%) in the plow layer at 0–25 cm because of high root density. On an area basis, the percentages of non-roots were somewhat higher at 0–25 cm (22%) and at 25–50 cm (30%). The higher area-based percentages were recorded because of larger diameter of non-root materials than of root material. The highest residue density at soil depths of 20–25 cm (1.17 $cm\,cm^{-3}$) indicated a plow depth of 25 cm.

4. Conclusions

Computer image analysis effectively discarded non-root debris from washed carrot root samples for very fine roots when residue length-to-width ratios were 3:1 or less. The data show that nearly invisible and short non-root materials can be separated by computer analysis better than by dedicated manual removal attempts. To separate large root images (corn) from organic residues, higher length-to width ratio ($\leq$15:1) was required (Dowdy et al., 1998). Additional research with a range of threshold ratios from 3:1 to 15:1 is needed to determine the optimum algorithm for agricultural crops of medium-sized root diameters under different cropping systems.

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