Measurement of Apple Root Losses Associated with Cold Storage and Elutriation of Soil Core Samples

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SUMMARY. Effects of washing and storing soil core samples of apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf. (syn. *M. domestica* Borkh. non Poir.)] roots were studied to determine root losses from processing samples. Root losses were assessed by measuring root lengths before and after elutriation and storage at 4 °C (39.2 °F). The accuracy of the automated root length scanner to measure individual fine roots [<1 mm (0.04 inch) diameter] of varying lengths was evaluated by first measuring roots, then cutting the roots into 2 to 3 cm (0.79 to 1.18 inch) lengths and rescanning. There was a significant relationship between the measurement of cut and noncut roots ($r^2 = 0.93$). Losses from elutriating samples with cut and noncut roots indicated a mean loss of

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50% for samples with cut roots and 34% for samples with noncut roots ($P \leq 0.01$). Total mean root loss (elutriation loss of noncut roots and degradation loss in cold storage) for the 12-month period ranged from 34% at month 0 to 53% at month 12 ($P \leq 0.01$). Mean root degradation losses from long-term cold storage ranged from 6% at month 1 to 19% at month 12 ($P \leq 0.01$). No losses were identified for roots with diameters of 1 to 5 mm (0.04 to 0.20 inch) and 5 to 10 mm (0.20 to 0.39 inch). A data correction curve was developed to correct root length data (<1 mm) for root losses associated with processing of soil cores.

One sampling method that is frequently used to study the spatial distribution of tree root systems is to determine the length of roots extracted from a series of soil cores (Mackie-Dawson and Atkinson, 1991). Coring requires the removal of a cylinder of soil, varying in size, from a designated area. Roots are extracted from the core by washing and the root length or mass is measured with root length being more frequently assessed (Mackie-Dawson and Atkinson, 1991). One of the major disadvantages of utilizing soil cores to study roots is the amount of time required to process large numbers of core samples.

The development of mechanical devices, such as power-driven coring machines to collect soil cores (Welbank and Williams, 1968) and hydropneumatic elutriators to extract roots from soil cores (Smucker et al., 1982), has greatly improved the speed and efficiency of collecting and processing core samples. Manual methods for measuring root lengths (Marsh, 1971; Newman, 1966; Tennant, 1975) have also been replaced with numerous automated techniques (Barnett et al., 1987; Farrell et al., 1993; Goubran and Richards, 1979; Harris and Campbell, 1989; Voorhees et al., 1980; Yanuka and Elrich, 1985). A Comair root length scanner (Commonwealth Aircraft Corp. Ltd., Melbourne, Australia) is one automated device that is based on the line-intercept method (Newman, 1966) and has been used with a high degree of accuracy for determining root length (Goubran and Richards, 1979; Richards et al., 1979).

Several problems may arise when

processing soil cores to determine root length. Root losses may occur when storing and washing soil samples. Depending upon the pore size of the screen used to collect root samples, many smaller roots may be lost when elutriating samples. Smucker et al. (1982) reported that hydropneumatic elutriators are efficient when using small pore size screens (#40), but nothing is known about the loss of roots through the larger pore size screens required for large soil samples with high silt and clay contents.

In addition, many soil samples are usually required for adequately determining root distribution of perennial crops. Even with automated techniques, it may not be possible to process all samples before root deterioration begins. To help preserve the roots until they can be processed, one approach is to maintain soil samples in cold storage [4°C (39.2°F)]. However, little information is known about the loss of roots from degradation over time in cold storage (Böhm, 1979).

The goal of this study was to identify and quantify apple root losses associated with elutriation and long-term cold storage of soil cores. This information will be used to develop a model of root loss over time and to correct root length measurements for samples collected in a long-term study on root distribution of apple trees.

Materials and methods

SAMPLE PREPARATION. Soil cores were extracted from orchards and adjacent, nonorchard areas in Turtle Creek Orchards in Kerrville, Texas. Sixty-one soil samples were extracted at 0.5 m (1.6 ft) from the trunks of irrigated, 5-year-old apple trees (*Malus sylvestris* var. *domestica* 'Fuji') on Malling 26 (M.26) rootstocks. An additional 61 samples were extracted from an area adjacent to the orchard without apple trees. Soil from all samples consisted of Nuvalde silty clay, a mixed thermic Typic Haplustoll. Soil was composed of 20% sand, 47% clay, 33% silt, and 2.8% organic matter with a pH of 7.9. Each sample consisted of a 10 cm (3.94 inch) diameter $\times$ 30 cm (11.81 inch) deep soil core extracted from the upper 30 cm (11.81 inch)

of soil. Soil cores were removed using a tractor-mounted Giddings Probe (Giddings Machine Co., Ft. Collins, Colo.) and immediately sealed in plastic bags and placed in cold storage (4°C) within 30 min.

The 61 samples taken near apple trees were elutriated using a hydropneumatic elutriator (Gillison's Variety Fabrication, Inc., Benzonia, Mich.) as described by Smucker et al. (1982). A US Standard #20 pore size screen was used to collect the roots to avoid plugging of a finer screen from the large, silty, clayey soil samples. Since melanized apple roots could be readily distinguished from other roots in the sample by their reddish brown color, they were separated from each sample and sorted by root diameter size. White root tips of apple trees were not collected. Apple roots were divided into three diameter classes [<1 mm (0.04 inch), 1 to 5 mm (0.04 to 0.20 inch), and 5 to 10 mm (0.20 to 0.39 inch)]. All the collected roots were combined based on root diameters and then divided into 61 samples containing similar numbers of roots from each diameter class.

Melanized roots (<1 mm) were measured on a Comair Root Length Scanner. Each sample was scanned three times to determine a mean length for the sample. The unit was factory calibrated, and samples were prepared

Fig. 1. Observed means (■, ●) and polynomial regression curves (solid lines, dotted lines) for elutriation root losses for noncut roots (■, solid lines) and cut roots (●, dotted lines). Polynomial regression equations are presented in Table 1.

Table 1. Formulas for polynomial and linear regression equations used in Figs. 1 to 5. In equations, M = number of months in cold storage, I = initial root length before elutriation, storage, and rescanning, and C = length of cut roots.

Fig.	Equation	r^2	P
1	Final root length after elutriation (noncut roots) = $-0.0103 \times I^2 + 0.879 \times I - 1.0006$	0.89	0.0001
1	Final root length after elutriation (cut roots) = $-0.0106 \times I^2 + 0.6188 \times I + 0.5959$	0.28	0.0487
2	Precut root length = $-0.0004 \times C^2 + 0.8892 \times C + 0.2255$	0.93	0.0001
3A	Total percent root loss over time = $-0.00137 \times M^2 + 0.02847 \times M + 0.35611$	0.43	0.0001
3B	Percent root degradation loss over time = $-0.00016 \times M^2 + 0.00995 \times M + 0.07529$	0.19	0.0120
4	Final root length after elutriation and cold storage = $0.5518 \times I$	0.65	0.0001
4	Final root length after cold storage = $0.7874 \times I$	0.68	0.0001
4	Final root length after elutriation (noncut roots) = $0.6677 \times I$	0.89	0.0001
5	Data correction multiplier for root length = $-0.0038 \times M^2 + 0.085 \times M + 1.5404$	0.43	0.0001

Fig. 2. Observed means (■) and linear regression line (solid line) for root length scanner measurements of root samples before and after cutting into 2 to 3-mm (0.08 to 0.12-inch) root pieces. Linear regression equations are presented in Table 1.

and scanned according to the root length scanner's instruction manual (Hawker De Havilland Victoria Limited, 1992). Melanized and suberized roots >1 mm were measured by hand.

ELUTRIATION LOSSES. Before scanning, roots (<1 mm) of 15 samples were cut into 2 to 3 cm (0.79 to 1.18 inch) pieces as recommended (Hawker De Havilland Victoria Limited, 1992), and the remaining samples (46 in number) were measured without cutting the fine roots. The measured roots (<1, 1 to 5, and 5 to 10 mm) were thoroughly mixed into the soil of the 61 samples that contained no apple roots. After incorporating the measured roots into the soil, the fifteen samples with cut roots and ten of the samples with uncut roots were immediately reelutriated, resorted, and remeasured to determine percent recovery of apple roots from soil cores after elutriation.

DEGRADATION LOSSES. Soil cores from 36 previously measured samples were stored at 4 °C. Each month three samples were elutriated, sorted, and remeasured. As in the initial measurements, the fine roots in each replicate sample were scanned three times to achieve a greater precision in measuring mean degradation. These measurements were compared with the initial measurements minus elutriation losses to determine root degradation during cold storage.

ROOT MEASUREMENT ACCURACY. Due

to the high root losses associated with elutriating cut roots, it became necessary to use noncut roots for the degradation experiment. Since roots were unable to be cut before scanning, an experiment was conducted to determine differences in the root scanner's measurements for cut and noncut roots. Twenty-five samples (<1 mm) were measured three times before cutting and then measured three times after being cut into 2 to 3-cm root segments to ascertain the accuracy and precision of using noncut roots.

DATA ANALYSIS. Analysis of variance was conducted using the general linear model procedure of the SAS statistical program (SAS Institute, 1992). Differences in total root losses

Table 2. Main effects of cold storage time on mean root lengths for root diameters <1 mm (0.04 inch); 1.00 m = 3.28 ft.^z

Cold storage time (months)	Mean root length		root loss ^w (%)
	Initial length ^y (m)	Mean Final length ^x (m)	
0	8.23	5.46	34.30 a
1	9.72	5.86	40.47 ab
2	6.81	---	---
3	7.66	3.87	48.14 bc
4	9.82	5.23	46.76 bc
5	8.73	4.83	45.07 abc
6	9.03	4.77	47.18 bc
7	9.33	4.79	47.54 bc
8	8.69	4.50	47.78 bc
9	9.56	4.83	48.69 bc
10	9.20	4.63	49.62 bc
11	7.88	3.82	50.65 bc
12	8.10	3.72	52.83 c

^zWithin criteria means followed by the same letter are not significantly different at $P \leq 0.05$, using least squares means procedure. Missing data (---) in month 2 denotes a processing error and was excluded from the analysis.

^yRepresents the mean final root length of three noncut root samples after elutriation, storage, and rescanning (samples = mean of three replicated measurements) except for storage time 0, which consisted of 10 samples.

^xRepresents the mean initial root length of three noncut root samples before elutriation, storage, and rescanning (samples = mean of three replicated measurements) except for storage time 0, which consisted of 10 samples.

^wRepresents the mean root loss for three samples [$1 - (\text{mean prestorage root length} / \text{mean poststorage root length})$] except for storage time 0, which consisted of 10 samples.

Fig. 3. Observed means (■) and polynomial regression curves (solid lines) for total root losses (elutriation and degradation losses) (A) and root degradation losses (B) over time in cold storage. Polynomial regression equations are presented in Table 1.

and degradation losses over time were analyzed, as well as losses associated with elutriation of samples with cut and noncut roots. Mean root losses were separated using least-squares means procedure (SAS Institute, 1992) with an α level of 0.05. Linear and polynomial regression were used to determine relationships between cold storage time and root loss, measurement of precut and postcut roots, and changes in initial and final root lengths associated with elutriating samples with cut and noncut roots. The means of the repeated measurements for each sample were used for analysis. Since there was a measurement error associated with the processing of the samples in month two, these data

were excluded from the analysis.

Results

ELUTRIATION LOSSES. Length of individual root segments (<1 mm) appeared to have a pronounced effect on the efficiency with which roots were recovered when elutriated. An average of 50% of the cut roots were lost as compared ($P \leq 0.01$) to an average root loss of 34% from the samples where the roots were not cut (Fig. 1, Table 1). There was also greater variability between the initial root length measurements and the root length measurements after the second elutriation for the cut roots ($r^2 = 0.28$) than the noncut roots ($r^2 = 0.89$) (Table 1). Based on these observations, further studies of root loss over time were conducted using noncut roots.

ROOT MEASUREMENT ACCURACY. The root length scanner demonstrated insignificant differences ($r^2 = 0.93$) between the measurement of fine (<1 mm) precut roots and cut roots (Fig. 2, Table 1). The scanner had a tendency to measure longer root lengths for cut roots than roots that were not cut. For the cut samples, 59% of the samples were measured by the scanner as having longer root lengths than the precut roots. The mean root length for all samples tested showed an average length of 5.3 m (17.4 ft) for the roots that were not cut and 5.8 m (19.0 ft) for the samples after they had been cut into 2 to 3 mm lengths ($P \leq 0.01$).

DEGRADATION LOSSES. There were slight degradation losses (Fig. 3A) of the fine roots (<1 mm) over the 12-month period. Total losses, including degradation and elutriation losses over the study period, ranged from an average of 34% at month 0% to

53% at month 12. Significant differences in total root loss over time are outlined in Table 2. When estimated losses due to elutriation were subtracted from the total losses, degradation losses ranged from an average of 6% at month 1 to 19% at month 12 (Fig. 3B). Significant relationships ($P \leq 0.01$) between the initial and final root length measurements for total root losses ($r^2 = 0.65$), root losses due to elutriation ($r^2 = 0.89$), and root losses due to degradation ($r^2 = 0.68$) were evident (Fig. 4).

Based on these data, a root length correction curve was derived to adjust fine root samples (<1 mm) for root losses from elutriation and cold storage (Fig. 5, Table 2). There were no measurable root losses or visible deterioration over the 12-month period for roots with diameters of 1 to 5 and 5 to 10 mm. After 24 months in storage, roots larger than 5 mm in diameter readily sprouted leaves when exposed to warmth and sunlight.

Discussion

Richards et al. (1979) demonstrated the measuring accuracy of an automated root length scanner by comparing root lengths estimated by the root scanner with actual root lengths. The optimum sampling range, which yielded the most accurate estimates, was from 20 to 50 m (65.6 to 164.0 ft) of

Fig. 4. Effects of elutriation, cold storage at 4 °C (39.2 °F), and elutriation and cold storage on lengths of apple roots in soil core samples in relation to theoretical samples with no losses. Linear regression equations are presented in Table 1.

Fig. 5. Correction curve, 95% confidence intervals, and 95% prediction intervals for adjusting root length measurements for root losses associated with elutriation and cold storage of soil cores over time. Polynomial regression equations are presented in Table 1.

roots per sample. Root samples containing less than 20 m of roots were usually overestimated. Low coefficients of variation between repeated measurements of the same sample demonstrated the precision of the instrument for measuring root lengths.

To optimize measurement results, it is recommended that individual root pieces need to be cut into 2 to 3-cm segments (Hawker De Havilland Victoria Limited, 1992). Smaller root segments provide for more even distribution of roots in the scanner tray, which decreases the chances of underestimating root lengths caused by clumps of overlapping roots. In addition, long roots tend to break through the surface of the water in the sample tray causing a meniscus of water around the root that can interfere with the scanner's ability to accurately measure root length. In our study, the differences between measurements of cut and noncut roots were negligible except for the tendency for cut roots to have longer root length estimates. This was probably due to poor distribution of noncut roots, menisci or both. The high correlation of measurements of cut and noncut roots allowed the study to be conducted using longer roots, which greatly decreased root losses from elutriation.

The loss of roots from elutriation of cut and noncut roots was significant. The actual loss from elutriation within

this experiment was probably underestimated because the roots had to be elutriated and measured before determining losses associated with elutriation. In addition, approximately one week was required to wash the samples, sort, measure the samples, and then reelutriate and remeasure them. Because of the processing time involved, it is likely that the elutriation losses also included some degradation losses as well.

Another concern of using the hydropneumatic elutriator is the significant differences in root losses based on root length. This could have implications when comparing root length densities between different studies. In this study, we used a 10-cm (3.94-inch) diameter coring tube. Other root studies of apples have been conducted with smaller [4 to 5-cm (1.57 to 1.97-inch)] diameter coring tubes (Hughes and Gandar, 1993). Roots from smaller diameter tubes may produce shorter root segments. Based on this study, elutriation losses from samples with shorter roots would be higher than longer roots from a larger diameter core. This may be offset by the fact that a larger diameter core takes longer to wash than a smaller diameter core of equal length, thereby increasing the time available for roots to be washed through the screen.

A substantial amount of time is required to process soil core samples from large studies designed to measure the spatial distribution of roots from trees. These studies are labor intensive and usually require large numbers of soil core samples that must be stored for some time period until processed. Although we have shown significant differences in losses of fine roots over a 12-month period due to degradation in cold storage, the practical differences are slight. When compared to other sources of root losses that occur when processing samples such as elutriation losses, root losses due to root degradation in cold storage are modest. Studies of roots >1 mm should expect insignificant degradation losses from cold storage of soil samples, while those focusing on smaller diameter roots (<1 mm) would need to develop correction factors with extended storage.

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