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## **ESTIMATING ROOT LENGTH DENSITIES AROUND TRANSPLANTED CONTAINER-GROWN PLANTS**

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**Abstract.** A simple method is described for measuring root length densities and is applied to the study of root distributions around transplanted container-grown plants. Roots were separated from core samples using a soil elutriation system and counted using Tennant's line intersect method. The elutriation system was constructed using inexpensive readily available materials. With this method a single operator can separate the roots from 40-50 samples per day. Soil core samples can be stored at 4 °C in plastic bags for at least three weeks and, after separation, in covered petri dishes for ten weeks without deterioration or fungal growth. Root lengths can be estimated for up to 80 samples per day. Root length density data from two *Escallonia rosea* shrubs are compared. One shrub was planted in unamended backfill soil and the other in amended backfill soil.

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The growth of the roots of a tree or shrub following transplantation is essential for establishment of the plant. To study establishment a convenient and accurate method for measuring the rate and extent of root growth after transplanting is necessary. Many different methods have been used for the study of plant root systems (1,3,15). Atkinson (1980) lists methods used for investigating tree root systems. These methods include whole tree excavation, glass wall or glass tube observation techniques, various methods of measuring root activity (i.e. radioactive tracers), and methods such as profile wall, monolith, and auger and core sampling.

Of these methods we believe that core sampling has great appeal for studying root growth both during establishment of new transplants and after plants have become established. To obtain soil-root samples auger or core sample has been used

successfully in several studies of the fine roots of trees (1,7,10,13,14,18,19,20). Core sampling is non-destructive and equipment required for sampling and for root separation is inexpensive compared to other methods.

Many different types of augers have been developed, including both hand augers and mechanical sampling machines (Bohm, pp. 39-47). Core sampling methods generally involve collecting a set of samples in the field and then separating the roots from the soil by washing in the laboratory. Several methods of separating roots from soil samples have been developed, ranging from washing roots by hand to flotation methods to various root-washing machines (Bohm, pp. 117-122) (4,5,6,8,16). Washing roots by hand is a tedious process and some of the more recent root-separation techniques have greatly facilitated the study of plant root systems.

After roots have been separated from the soil, root growth can be expressed, depending on the aims of the study, as weight, surface area, volume, diameter, length or the number of root tips (Bohm, p. 125). In recent years an increasing number of studies have used root length density (root length per unit soil volume) as a measure of root growth. This is a very useful parameter because root length density is closely related to water and nutrient uptake by the plant.

To estimate root length density, total root length per sample must be known. Since very extensive root lengths can occur in small volumes of soil, it may be impractical to directly measure all the roots in a sample. Tennant (1975) has described

a modification of Newman's (1966) line intersection method whereby total root length is estimated by counting the number of intersections of roots with the lines of a grid. According to Tennant, root length =  $11/14 \times \text{number of intersects} \times \text{grid unit}$ . In the present study a core sampling method to estimate root length density around transplanted container-grown trees and shrubs was developed. This report discusses a procedure for sampling, root separation, and counting to obtain root length density estimates.

### Methods

**Sampling.** The soil auger used to obtain core samples was the "Lord Sampler" (Soil Moisture, Inc.) which consists of a semi-cylindrical coring tube with an inside diameter of 1.9 cm. The tube is marked at 15 cm intervals and samples can be taken to a depth of one meter.

In this study core samples were taken as a function of depth and distance from the plant crown of two *Escallonia rosea* shrubs. The shrubs were transplanted from 1 gallon containers during the spring of 1981 into a Yolo loam at Davis, California. At the time of planting, the hole for one of the shrubs was backfilled with the excavated loam soil while the hole for the other shrub was backfilled with soil amended 25% (by volume) with fir bark amendment. Soil cores (15 cm long) were taken during the winter of 1983. Each sample was a composite of three cores giving a total volume of 130 cm<sup>3</sup>. Samples were taken from the north, south, east and west sides of each plant at 13, 25, 38 and 50 cm radii from the plant crown and at 1-15, 15-30 and 30-45 cm depth intervals. Samples were placed in small plastic bags and stored at 4°C. Under these conditions the roots remained fresh for several weeks until the samples could be processed.

**Separation of roots from soil samples.** The method used to separate the roots from the core samples of known bulk volume was similar to the hydro-pneumatic elutriation system described by Smucker et. al. (1982). To disperse the soil various techniques including pre-soaking the sample and addition of dispersing chemicals have been used to facilitate root washing (3,6,16,20). In the present study neither pre-soaking nor the use of dispersing agents was necessary. The soil

was dispersed by placing a core sample in an Osterizer® blender along with about 500 ml of tap water and agitating the sample at the slowest speed for a period of 20-30 seconds. To prevent root shredding the blades of the blender were covered with Tygon® tubing.

The dispersed soil containing the roots was transferred to a lucite tube elutriation chamber, illustrated in Fig. 1. Following separation the roots

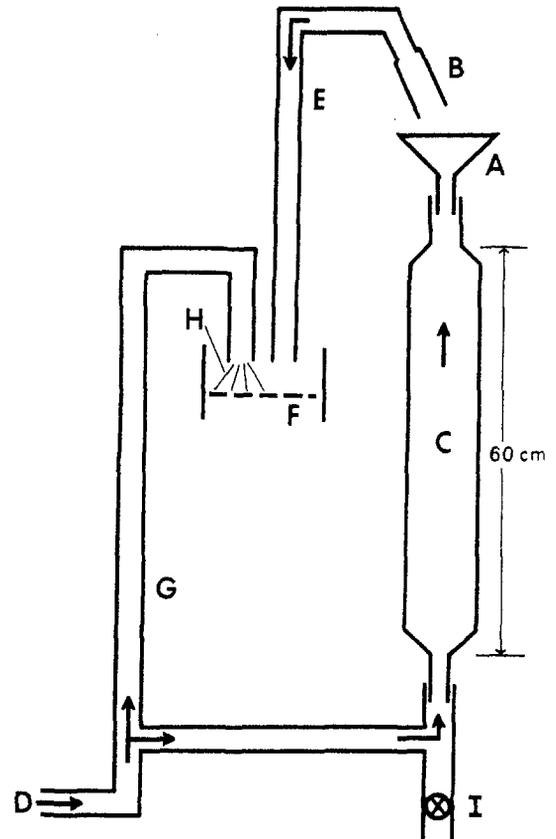


Fig. 1. Schematic diagram of the root-soil separation system. The dispersed sample is poured into the elutriation chamber through the funnel (A). The funnel is removed and B is friction-fit to the elutriation chamber (C). Water pressure is then applied (D) so that roots float upward through the elutriation chamber and through the flexible Tygon tubing (1.25 cm I.D.) (E) and are expelled onto a 40-mesh screen (F). Water simultaneously flows through the high-pressure rubber tubing (G) and is emitted as a jet spray (H) which prevents fine soil particles from plugging the screen. When roots are no longer being collected on F the coarse soil particles and coarse roots remaining in the elutriation chamber are released through the check valve (I) onto another 40-mesh screen.

on both screens were transferred to a beaker, and by decanting, the roots could be separated from coarse soil particles and from coarse organic amendment particles. The roots were then collected on a 15 cm diameter Whatman #1 filter paper by vacuum filtration. The filter paper containing the root segments was then placed in a covered petri dish. With this method a single operator can separate the roots from the soil of 40-50 samples per day.

**Estimation of root length density.** The line intersect method of Tennant (1975) was used to estimate root length utilizing the formula: root length in cm =  $11/14 \times$  number of intersects  $\times$  grid unit in cm. Root length density in  $\text{cm}/\text{cm}^3$  was computed by dividing root length by volume of the sample. Preliminary tests revealed that a 1 cm grid unit gave us the best combination of precision and speed of counting. The grid was prepared on a clear acetate disc (15 cm diameter) using a photocopying machine and was attached to the bottom of a plastic petri dish. When storing a sample, the filter paper containing the root segments was placed in the inverted top of the petri dish with the inverted bottom half placed over the roots as a cover. For counting, the 1 cm grid was placed on top of the roots. The number of intersections between the roots and the grid lines was counted with the aid of a binocular microscope and a hand tally counter. Live roots could easily be differentiated from dead roots and from organic amendment particles but counting was slowed considerably for samples with large amounts of organic matter. Standard deviations obtained with repeated countings of a single sample were about five percent. With this method a single operator can estimate root lengths for 60-80 samples per day, depending on the root densities and amount of organic amendment in the samples.

## Results and Discussion

Root length densities around the transplanted *Escallonia* plants are shown in Fig. 2. Root length density varied from 0.3 to  $2.5 \text{ cm}/\text{cm}^3$ . This range agrees closely with that reported by Atkinson (1980) for tree species. At the time of planting, the container root balls were 15 cm in diameter by 15 cm deep and the planting holes were 40 cm in diameter and 15 to 20 cm deep. The core

samples taken at the 13 cm radius and 0-15 cm deep were thus in the region of backfill containing organic amendment for one plant but not for the other. (Fig. 2). Samples taken at the 25 cm radius were just beyond the edge of the original planting hole.

For the plant growing in unamended backfill the mean root length density at the 13 cm radius and 0-15 cm depth was about half the mean density at the same location for the plant growing in amended backfill (Fig. 2). The root length density, however, at the 13 cm radius and at the 30-45 cm depth, well below the backfill region, was about three times greater for the plant in unamended backfill than it was for the plant growing in amended backfill. The fir bark amendment apparently caused a two-fold increase in root branching in the amended region, but the number of roots extending to a greater depth was significantly reduced. For both plants the root length densities at the 50 cm radius were very low and were virtually identical at all three depth intervals.

**Variability in root distribution and root growth.** Considerable variability in root length density was found between samples taken from a single plant at the same radius and soil depth (Fig.

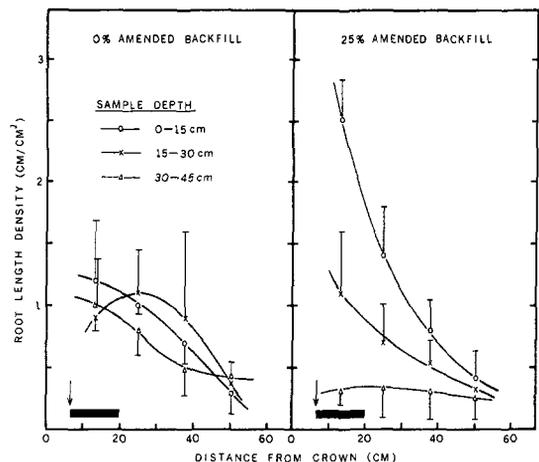


Fig. 2. Root length densities around transplanted container-grown *Escallonia rosea* shrubs planted in unamended and amended backfill. Core samples were taken from four different sides of the plant and vertical bars represent one standard deviation. Arrow at 7.5 cm indicates edge of original root ball. Horizontal solid bar represents width of backfill around root ball.

2). This same variability has been observed in other studies in which core sampling was used (2,10,13,20). There are several possible explanations for this variability. Root length density can be greatly influenced by variation in soil properties such as texture, structure, moisture, and aeration (12, 18). Higher root densities within a particular part of the root system may be due to enhanced lateral branching under optimal local soil conditions. With container-grown plants different numbers of roots could also be originating from different locations of the root ball.

**Storing root samples.** To determine how long the washed root samples could be stored in covered petri dishes at 4°C, samples were treated with about 1 ml of deionized water or one of 3 concentrations of bleach solution at the time of separation. Root lengths were estimated at two week intervals, and after ten weeks, the roots still appeared fresh with little or no fungus growth and the root length density estimates remained virtually unchanged. Since core samples can be stored at 4°C for at least three weeks before being separated and then again for ten weeks before the roots need to be counted, there is considerable flexibility in scheduling of operator work time.

The data shown in Fig. 2 were obtained from plants which had gone through two growing seasons. Work is in progress in which root growth of container-grown trees and shrubs will be measured during the first growing season following transplantation. It is this period which is most critical in the establishment of the plant. By taking core samples at various lengths of time after planting, the method described here should be a convenient way to study the rate of root extension and early root distribution patterns around a transplanted container plant.

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