Volume Displacement Provides a Quick and Accurate Way To Quantify New Root Production

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Root growth potential (RGP) measurement can be time-consuming and tedious, especially when seedlings have fibrous root systems. A volume-displacement technique involving suspending seedling roots and shoots in a clear plastic tube allows rapid estimation of the volume of plant parts while minimizing gravimetric errors. The technique was tested in three experiments examining (1) the repeatability of the technique, (2) its usefulness in estimating RGP, and (3) the relationship of volume displacement to tissue dry weight. Results indicate that this technique provides a quick, reproducible measure of seedling size while allowing a rapid (2-seedlings-per-minute) assay of RGP in container-grown stock. Tree Planters' Notes 45(3): 121–124; 1994.

Seedling morphological attributes have been used extensively in reforestation research to assess seedling performance potential and to explain outplanting performance (Mexal and South 1990, Mexal and Landis 1990). Nondestructive measures of seedling morphology allow for measurement of the entire test population. This increases the sensitivity of analysis by eliminating the need for subsampling. Traditional nondestructive measures include seedling height, root collar diameter, and (occasionally) total seedling fresh weight. In addition to providing these measures, volume displacement analysis allows total biomass to be subdivided into shoot and root volume. Volume displacement analysis provides sensitive and repeatable morphological information that may be useful in physiological analysis of seedling performance potential (for example, in root growth potential analysis) (Burdette 1979).

There are two approaches to volume displacement analysis. The first approach, actual volume displacement, measures the volume of water displaced when plant tissue is submerged in a vessel of water (Novoselov 1960). The second is a gravimetric approach based on Archimedes' principle, which states that "a body wholly or partly immersed in a fluid is buoyed up by a force equal to the weight of the fluid displaced" (Weast 1980). In the gravimetric approach, change in weight is used as the estimate of plant volume. A system for using gravimetric volume displacement is described below (see Materials and Methods).

Volume displacement analysis has the advantage of providing a fast measure of new root production (Burdette 1979). In addition, its nondestructive nature permits repeated measures over time. However, previously published volume displacement techniques have serious limitations. First, techniques that measure actual volume displaced require elaborate glassware configurations if they are to be sensitive enough to detect slight differences in seedling stock. Second, most gravimetric approaches require balancing the seedling in the water vessel at a specified point on the seedling so that plant tissue submerged in the water does not touch vessel walls (Burdette 1979). If the plant touches the container wall, the balance may fail to provide a steady reading, or the plant's frictional resistance may cause tissue volume to be underestimated. Any slight adjustment in holding the seedling may produce erroneous measures, compromising the accuracy and repeatability of the experiment.

In three experiments, a technique was tested for determining plant part volume and for quantifying new root production. This technique was designed to be simple and repeatable, using common laboratory equipment.

Materials and Methods

Gravimetric technique. The gravimetric technique uses several common laboratory devices (figure 1): a top-loading balance weighing to the nearest milligram; a 1,000-ml graduated cylinder filled with water; a clear plastic tube suspended in the graduated cylinder to support the seedling and prevent it from touching the walls of the graduated cylinder; and a ring stand and clamp to support the plastic tube.

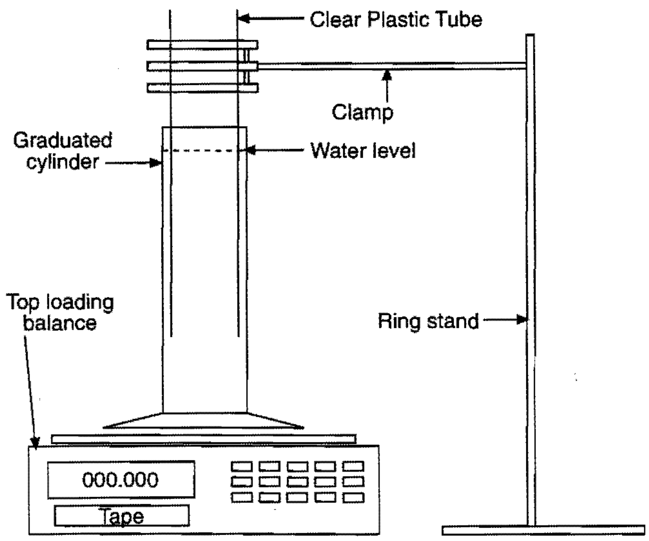

Figure 1A—*Schematic of gravimetric apparatus used for volume displacement analysis.*

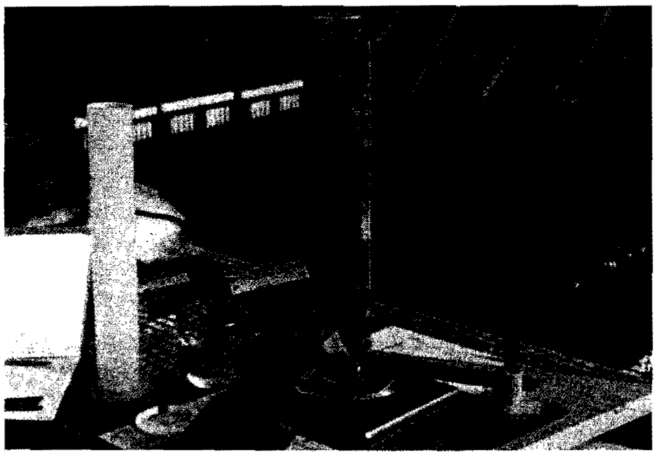

Figure 1B—*Close-up of graduated cylindar in gravimetric apparatus.*

Before volume measurement, seedlings were removed from their containers, and their root balls were rinsed free of medium under running water. The washed root systems were blotted dry. Then the balance weight was set to zero and the seedling tissue to be measured was placed into the water inside the plastic tube. To determine root system volume, the seedling was submerged until the surface of the water was 2 mm (0.08 in) above the uppermost lateral root (the cotyledon scar can also be used). This provided a reference point on the seedling for repeated measurement, thereby reducing experimental error. To determine shoot system volume, the seedlings were inverted and submerged until the surface of the water was 2 mm (0.08 in) above the base of the lowermost foliage (the cotyledon scar can also be used). Following immersion of the plant part to be measured, the new balance reading was recorded and used as an estimate of plant part volume.

Plant material. Container-grown eldarica pine (*Pinus eldarica* Medw.) and Arizona cypress (*Cupressus arizonica* Greene) were used in this study. Seedlings were grown for 20 weeks in Ray-Leach Super Cells® (164 cm^3; Ray-Leach Corporation, Oregon) containing a 2:1:1 (v/v/v) peat–perlite–vermiculite mixture. Seedlings were grown at the New Mexico State University Forestry Greenhouse located in Las Cruces, New Mexico. Ambient light was supplemented by 100-W incandescent bulbs to maintain a 16-hr photoperiod. Greenhouse temperatures ranged from 18 to 27 °C (64 to 81 °F) during the day to 16 to 23 °C (61 to 73 °F) at night. Seedlings were fertilized weekly with 15 ml of a modified Hoagland's nutrient solution (109 ppm nitrogen, 29 ppm phosphorus, and 17 ppm potassium).

Experiment 1. The first experiment examined the repeatability of the volume displacement technique. Root and shoot systems of the 20 pine seedlings over a range of sizes (based on shoot height) were measured 5 consecutive times using the volume displacement technique described above. Between measures, seedlings were blotted dry with paper towels. From this information, a mean value and coefficient of variation were calculated for the root and shoot tissues of each seedling.

Experiment 2. The second experiment examined the relationship of volume displaced to the tissue dry weight of seedlings measured. Root and shoot system volumes were determined for 48 eldarica pine and 19 Arizona cypress seedlings using the volume displacement technique described above. Seedling root and shoot systems were then separated and placed in a drying oven at 65 °C (149 °F) for 48 hours. After drying, the seedling tissues were removed and weighed to the nearest milligram. Then the tissues were returned to the oven for an additional 24 hr and reweighed. No difference in dry weight measurements was found for the 48- and 72-hr treatments. From this information, regression analysis was used to examine the relationship between volume estimation and tissue dry weight.

Experiment 3. In the third experiment, the volume displacement technique was evaluated for its utility in root growth potential (RGP) evaluation. Root system volumes of 30 eldarica pine seedlings were determined using the volume displacement technique described above. Seedlings were then placed in an aeroponic rooting system (Rietveld and Tinus 1987). After 14 days of incubation, seedling root systems were blotted dry and the volume measured again. New root volume was determined by subtracting the initial root volume from the root volume following RGP incubation. In addition, new roots greater than 0.1 cm (0.04 in) in length and greater than 0.5 cm (0.2 in) in length were counted for each seedling. From this information, regression analysis was used to determine the correlation between change in root volume and number of new roots greater than 0.1 cm (0.04 in) and 0.5 cm (0.2 in) in length.

Results

Experiment 1. This technique is repeatable, with coefficients of variation that are quite low for this type of measure, ranging from 0.22 to 2.00% for shoot tissue and 0.34 to 1.95% for root tissue (table 1). There was no apparent trend in variability based on tissue size.

Experiment 2. In experiment 2, strong relationships between volume displaced and tissue dry weight were found for shoot and root tissues of both species (figures 2 & 3). Linear correlation coefficients (r) for the relationships ranged from 0.91 for eldarica pine root tissue to 0.98 for Arizona cypress root and shoot tissues.

Experiment 3. In the third experiment, the increase in new root volume after the 14-day test period ranged from 5 to 50%. Most seedlings had at least a 10% increase in root volume, which was well above the coefficients of variation found in the first experiment (CV = 1.95%). The majority (mean = 75.8%) of the new roots produced during the RGP incubation were between 0.1 cm (0.04 in) and 0.4 cm (0.16 in) in length. The relationship between new root volume and number of new roots greater than 0.1 cm (0.04 in) had a

Table 1—*Size range of eldarica pine seedlings and coefficient of variation range for volume displacement technique*

Tissue	Size range (g)	CV range (%)
Shoot	3.90–7.60	0.22–2.00
Root	2.66–5.50	0.34–1.95

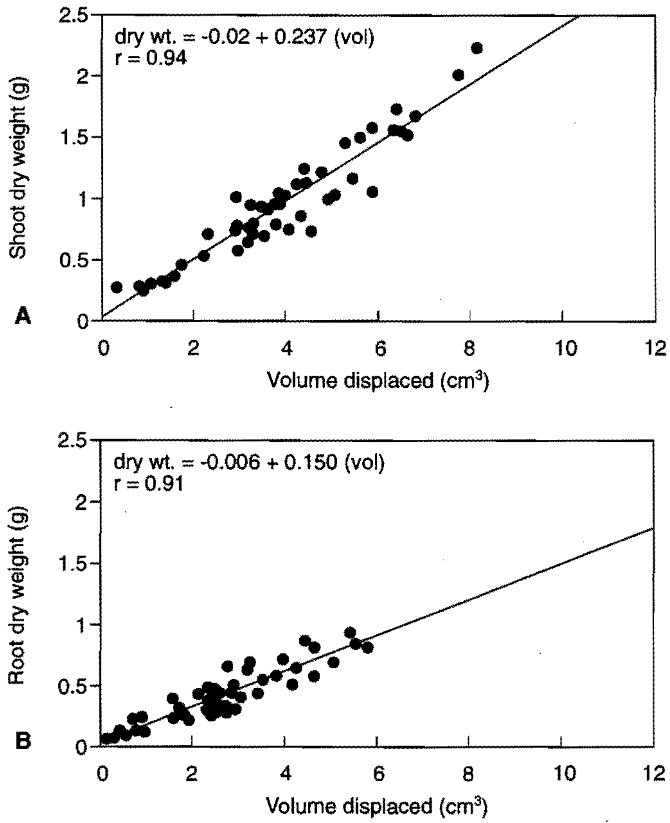

Figure 2—*Relationship between volume displaced and tissue dry weight for eldarica pine seedlings. Shoot tissue (**A**) and root tissue (**B**) are measured separately. Solid line represents fitted regression equation.*

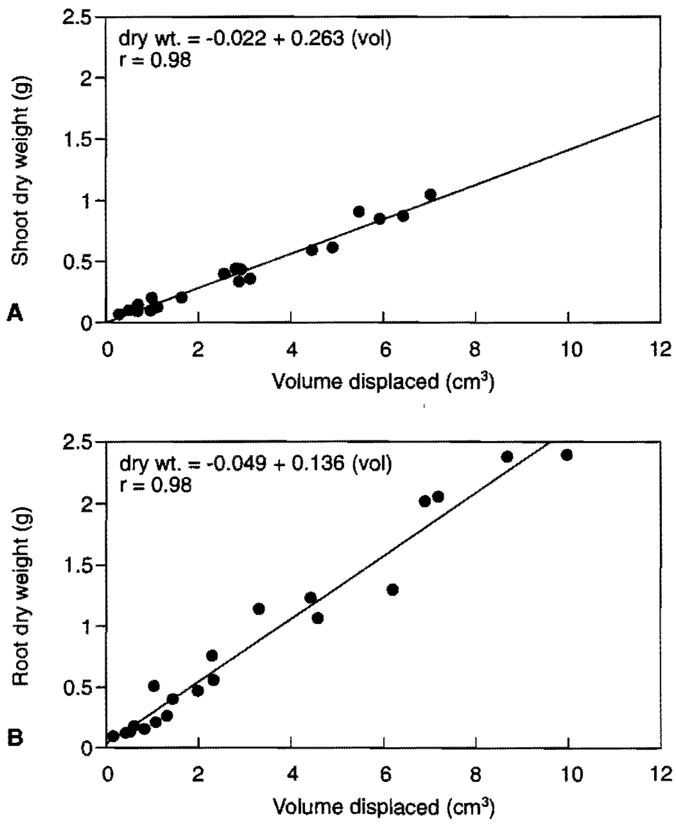

Figure 3—*Relationship between volume displaced and tissue dry weight for Arizona cypress seedlings. Shoot tissue (**A**) and root tissue (**B**) are measured separately. Solid line represents fitted regression equation.*

linear correlation coefficient of 0.80 (figure 4). The relationship between the number of new roots greater than 0.5 cm (0.2 in) and new root volume was not as strong, with a linear correlation coefficient of 0.53.

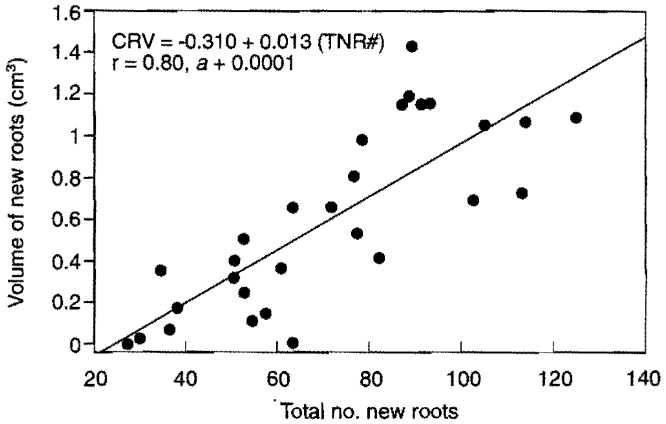

Figure 4—*Relationship between new root number and new root volume for eldarica pine seedlings. Solid line represents fitted regression equation.*

Discussion

This technique provides a nondestructive, repeatable approach to evaluating seedling morphology. Unlike previous gravimetric techniques (Novoselov 1960, Burdette 1979), this approach reduces emphasis on experimental mechanics (such as balancing the seedling and holding it steady) by using the plastic tube in the container (a "tube within a tube") to eliminate frictional resistance of plant tissue against container walls (see figure 1). Due to its nondestructive nature, this gravimetric technique allows for repeated measures through time on the test population, a decided advantage in RGP analysis. Moreover, it is simpler and faster than other techniques of assessing new root growth. Previous RGP studies have measured increases in root system using root area meters (Rietveld 1989), which can be expensive and difficult to calibrate. With stock that has fibrous root systems, root counts taken in most RGP studies can be very time-consuming. It can take up to 5 minutes per seedling to count new roots in container-grown stock, and 10 minutes per seedling to measure new root length. By contrast, the gravimetric technique evaluated here can monitor new root production at a rate of 2 seedlings per minute, permitting larger sample sizes to be tested if needed.

In the third experiment, the poor correlation of volume displaced with the traditional measure of new root growth (roots longer than 0.5 cm, or 0.2 in) is related to the much greater frequency of new roots that were shorter in length. On average, roots ranging from 0.1 to 0.4 cm (0.04 to 0.16 in) in length constituted 75.8% of new roots per seedling.

Conclusions

This gravimetric technique has several applications in root growth analysis. First, it obviates the need for subsampling in studies of the developmental process of new root production and reduces the impact of the measurement process on seedlings. Second, it can be used to relate new root production to previous root volume (Burdette 1979). Finally, it can be used to obtain morphological measures potentially useful in RGP tests that employ covariates to reduce sampling error, as suggested by South and others (1989).

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