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A rootbox for quantitative observations on intact entire root systems

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Abstract

A rootbox is described which allows observation of an intact, entire root system. Roots are sandwiched against a plexiglass surface by a nylon mesh that is impermeable to roots, but permeable to water and nutrients. To quantify root growth non-destructively, roots of different size classes are traced onto acetate sheets using different color pens, and root lengths determined by digital image analysis.

Introduction

Differences in root growth patterns have been found between roots at observational faces of rootboxes and rhizotrons and those in soil behind the faces (Taylor and Bohm 1976; Voorhees 1976; Voorhees *et al.* 1980), and may hinder attempts to quantify experimental effects on various aspects of root growth, such as length, surface area, biomass, longevity and nutrient uptake. For experiments involving mycorrhizae, different growing conditions may make interpretation of results equivocal.

To avoid some of these problems, we have developed a method for growing roots that allows observation of the growth of the entire root system, and that is amenable to accurate quantification. In this paper we (1) describe the construction and use of a rootbox which keeps all root growth visible and (2) describe how digital image processing can be used for non-destructive measurements of the increase in root length for roots of differing size classes.

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Materials and methods

A rootbox similar in design to that described by James *et al.* (1985), but developed independently, was built out of 0.318 cm clear plexiglass (Fig. 1). Each face was 20.3 × 35.6 cm. Plexiglass spacers (1.3 cm) were used to separate the faces, and threaded bolts were placed along the edges to hold the rootbox together. A spacer with holes was also placed along the bottom to allow drainage. Rubber gaskets were used along the spacers to provide an airtight seal around the rootbox.

To permit observation of the entire root system, the roots were grown in a plane between the plexiglass surface and a nylon sheet (28 um pore size, Tetko, Elmsford, NY) that separated roots from the soil medium, which in this case was vermiculite. A foam strip along the top helped hold the nylon in place, and also prevented soil particles from getting between the nylon and plexiglass surface. Two seedlings of slash pine (*Pinus elliottii*) were grown on opposite sides of each rootbox, thus effectively

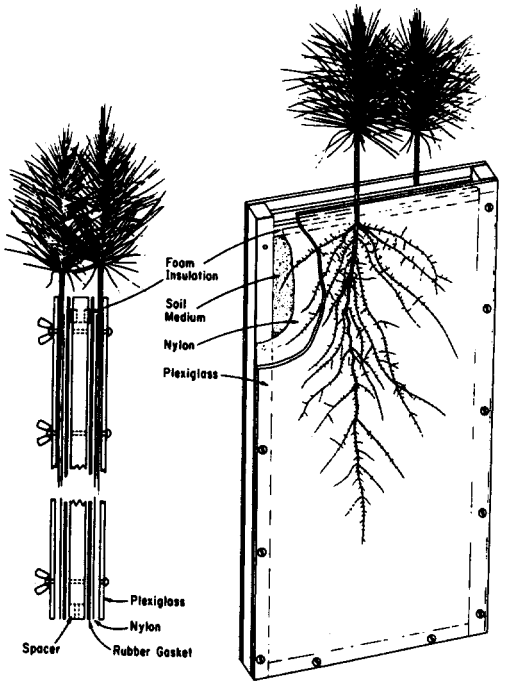

Fig. 1. Rootbox used for growing trees: side view and frontal 3-D view. See text for details on size and materials used.

doubling the sample size with little or no increase in space requirements. Several of the rootboxes were placed in a wooden box and thick closed-cell foam insulation was placed between each to keep light from the root systems.

Plants received water whose chemical composition was determined primarily by the chosen nutrient solution and to a lesser degree by the soil medium, similar to the soil-less growing method described by Cappy and Brown (1980). Snow and Tingey (1985) used the same type of nylon cloth to prevent root escape in water stress units and obtained excellent growth using a number of plant species. No evidence was found for root hair penetration through the nylon with the seedlings used in the present study, but it can occur in some species (Hartley, pers. comm.).

In order to assess the suitability of our rootboxes for growing plants, comparisons were made of several growth parameters between the slash pine seedlings in the rootboxes and those growing in pots under similar environmental conditions. Plant height and diameter just below the cotyledons were measured for plants in the rhizotrons at 4–5 day intervals, while at the conclusion of the growth period, plants from both the pots and rhizotrons were harvested, dried to constant weight at 65°C, and then weighed. Subsamples were ground in a Wiley Mill and analyzed for nutrient concentrations using inductively coupled plasma emission spectrometry.

For non-destructive quantification of root growth we adopted the following procedure. First, after each seedling was established, and new root growth had resumed (typically about 1 week after transplanting) we traced the entire root system onto clear acetate sheets with permanent markers. We divided the root system into three size classes: 1 mm or less, 1 to 2 mm, and larger than 2 mm (usually just the tap root). Different color markers were used to draw roots of each size class. We recorded the increase in root length for each of the size classes at 4–5 day intervals by re-positioning the previous trace and drawing only those roots that had grown since the previous measurement. A typical tracing took about 15 minutes.

We used digital image analysis to quantify initial root lengths and root length increases from the tracings. A backlit transparency of each tracing was digitized using a microcomputer-based image processing system.

Three separate imaging routines were compared for their accuracy and speed in estimating initial root lengths and growth. The first routine consisted of retracing the roots using a mouse-driven cursor. The second routine automatically followed edges of root tracings, calculating root length as the perimeter divided by two. Different color filters were used to distinguish the various root size classes. A third method simply involved measuring the total area of the tracings (again using color filters to separate size classes), and calculating length as total area divided by the average trace width.

Results and discussion

Slash pine seedlings grew vigorously in these rootboxes, and mycorrhizae readily colonized and infected the roots. Root hairs developed normally on slash pine (Fig. 2a) and Ponderosa pine (Fig. 2c) seedlings grown in a slightly different chamber (see Rygiewicz *et al.*, 1988) for a description of this particular chamber design). Typical club-root development resulted from mycorrhizal infection

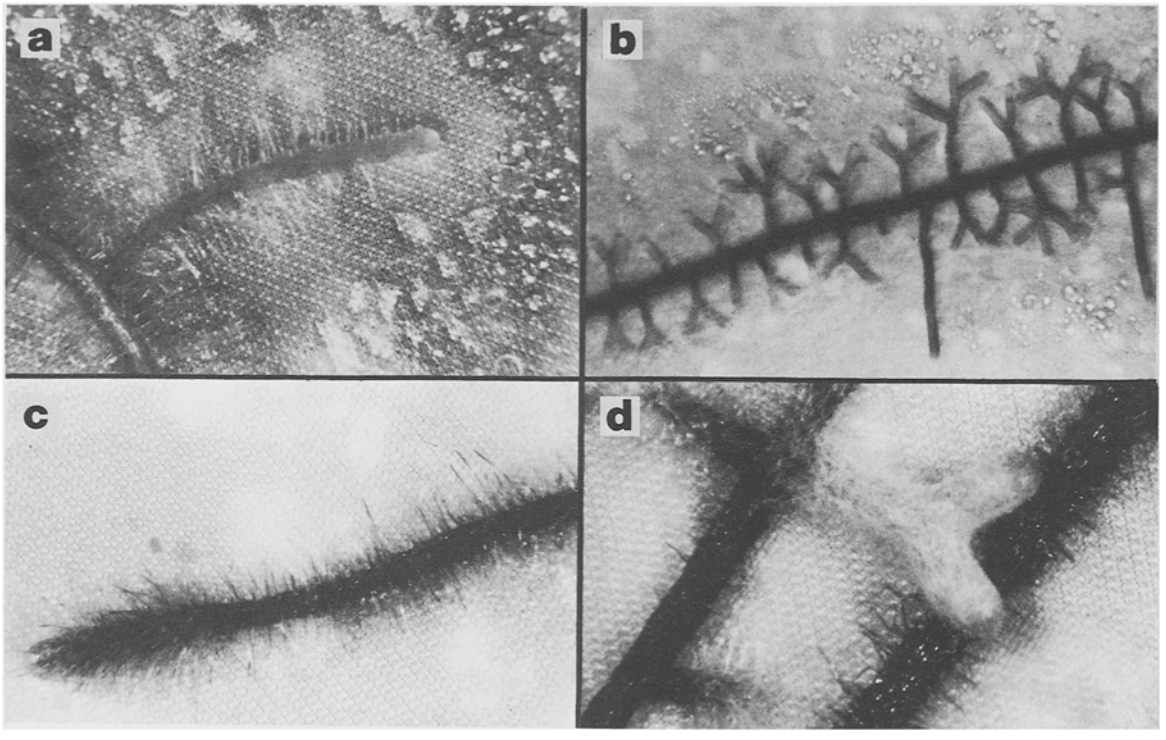

Fig. 2. Roots of slash pine (*Pinus elliottii*) and Ponderosa pine (*Pinus ponderosa*) produced in the rootboxes and grown between the plexiglass surface and the nylon mesh: **a** slash pine root hairs; **b** slash pine roots infected by mycorrhizae (not identified); **c** ponderosa pine root hairs; **d** ponderosa pine roots infected by the mycorrhiza *Laccaria laccata*.

(Figs. 2b, 2d). In later experiments, boxes similar in design to those described by Rygiewicz *et al.* (1988) have been modified to grow roots against the nylon but which allow for their removal (without undue disturbance to the plant) and subsequent assaying for ^{14}C and starch in root tissue.

Mean seedling height increased from 1.5 cm to 5.5 cm, while mean diameter increased from 1.24 mm to 1.98 mm. Root-shoot ratios were not significantly different between potted and rhizotron plants as determined by a t-test (0.396 ± 0.033 vs 0.379 ± 0.011, $n = 5$ and $n = 8$, respectively, $p > 0.05$). With only a few exceptions (Al, Mn), rootbox seedlings generally had greater tissue nutrient concentrations (data not shown).

Among the three software routines, the most accurate technique was to retrace the system using a mouse-driven cursor, essentially the technique of Belgrand *et al.* (1987). The results are shown graphically in Figure 3. Note that most of the new root growth occurred in the smaller roots. With this method, length was estimated to within 2% of that measured by hand, but took the longest (29 mins/root system). The edge technique was less accurate, but could be done more rapidly. Small roots were often overestimated with this technique, mainly due to the low length to width ratios and irregularities in the tracings which inflated the perimeter values. The area technique proved fairly accurate (within 10%) as long as it was calibrated against each root tracing. Failure to calibrate resulted in underestimates of length because average trace widths tended to be overestimated.

In conclusion, the rootbox described in this paper can be used to grow plants with root systems fully visible for destructive or nondestructive measurements of growth and physiology. We have also described simple image processing methods (available on request from P.M.R.) that can be used to rapidly quantify the growth of intact root systems.

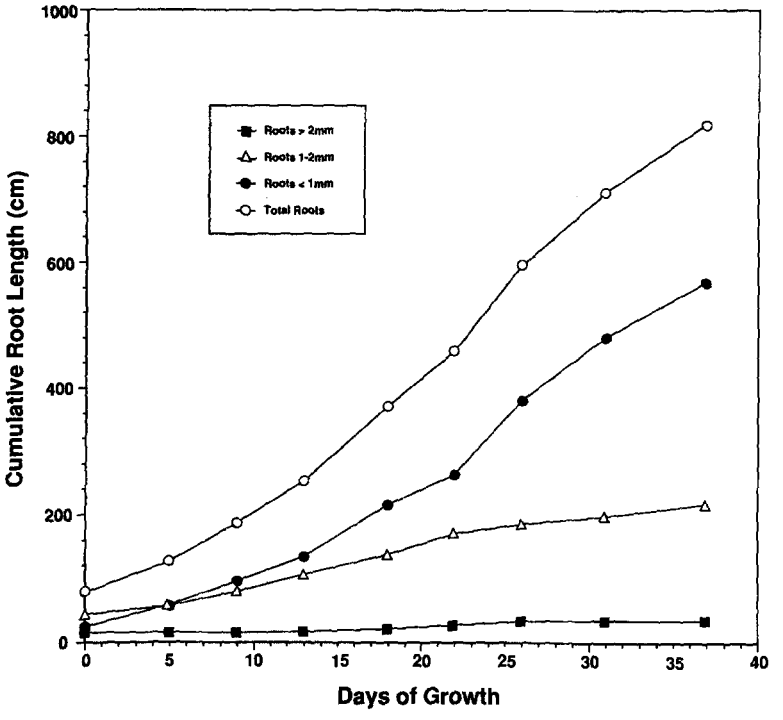

Fig. 3. Root growth as a function of time for each of three diameter size classes in a typical slash pine (*Pinus elliottii*) seedling. Values are those obtained from digital image analysis, tracing routine.
Legend:- ■ Roots > 2 mm; △ roots 1–2 mm; ● roots < 1 mm; ○ total roots.

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