An Inexpensive Rhizotron Design for Two-Dimensional, Horizontal Root Growth Measurements

Adam H. Wiese, Don E. Riemenschneider, and Ronald S. Zalesny, Jr.

Forestry Technician, Project Leader (Research Plant Geneticist), and Research Plant Geneticist; USDA Forest Service, North Central Research Station, Forestry Sciences Laboratory, Rhinelander, WI

Abstract

We designed, constructed, and tested an observational system that supports two-dimensional, horizontal root growth measurements over time without disturbing aboveground plant growth and without the need for destructive sampling of roots. Our rhizotrons allow for (1) studying relatively greater numbers of plants at any given time than is now possible under traditional technologies in a crop development context, (2) observing the horizontal orientation of root systems, which ultimately supports the study of competition among crop trees and between crop trees and invasive weed species, and (3) acquiring novel rooting data that can be input to a plant growth model. Our system is primarily constructed using common materials such as plexiglass and aluminum channel at approximately $375.00 per unit.

Introduction

Aboveground growth of many trees has been studied extensively (Gower and others 1993; Wu and Stettler 1994; Orlovic and others 1998). In contrast, study of root growth has been limited because of the difficulty of acquiring meaningful data, the cost associated with excavation, the logistics associated with accessibility, and limited efficiency of sampling procedures (Carlson 1965; Lauenroth and Whitman 1971; Friend and others 1991). Knowledge of the growth and spatial orientation of roots can be valuable to better assess plant responses to a wide variety of factors, including but not limited to genetic effects, fertilization, animal browsing, herbicide application, and climatic conditions. Joint knowledge of aboveground and belowground growth could lead to development of technologies supporting increased plant yields and greater overall health of the plant community.

We have developed a new method of studying root growth and development over time without disturbing aboveground plant productivity (Kokko and others 1993; Stoermer 1996; Kaspar and Ewing 1997). One of the major problems often associated with rooting research is the collection of fine roots from the planting medium, which is tedious and may result in underestimates because of loss of fine tissue (Bohm 1979; Heilman and others 1994). Another problem is the inability to view a large area in which the roots are contained, while leaving the plant undisturbed in order to observe growth responses over extended time periods (Newman 1966; Yorke 1968).

As a result of the aforementioned problems, existing systems for studying belowground root growth failed to meet our needs. Our objectives were to (1) observe relatively greater numbers of plants at any given time than is now possible under traditional technologies in a crop development context; (2) observe root systems from a two-dimensional, horizontal orientation so that we can ultimately study competition among crop trees and between crop trees and invasive weed species; and (3) acquire novel rooting data that can be input to a plant growth model (i.e., measurements of root geometry over time). The following sections provide detailed plans and steps describing construction of our rhizotron, along with a summary of the kind of data that can be collected while using the rhizotron.

Rhizotron Construction

Refer to figure 1 for a photograph of operational rhizotrons.

Materials/Equipment. The rhizotrons cost approximately $375.00 each, with 0.25-in- (0.64-cm-) thick plexiglass accounting for the majority of the total cost (approximately $159.00/sheet). Our rhizotrons supported six equally divided compartments within a 4- x 8-ft (1.219- x 2.438-m) sheet of plexiglass; however, the rhizotrons are versatile enough to accommodate other spacing systems, based on specific experimental objectives. In addition, we

Figure 1. *Operational rhizotrons used for two-dimensional, horizontal root growth measurements. Observations of the root systems are taken on the underside of the rhizotrons by removing the tarps.*

built a rhizotron framework consisting of a 2- x 4-in (5.08- x 10.16-cm) outer frame with a 1- x 4-in (2.54- x 10.16-cm) inner frame to support the weight of the rhizotron, which was approximately 80 lb (36.287 kg) when complete. The rhizotron framework may be optional if your greenhouse table can support the weight load and still allow working room under the rhizotrons to view root development. A support framework constructed with 1.25-in (3.175-cm) galvanized steel pipe held the rhizotron and its framework above the ground, while leaving adequate space under the rhizotron to view root growth. Table 1 provides a list of equipment and materials needed to build the rhizotron, rhizotron framework, and support framework, according to our spacing system.

Rhizotron Assembly. Figure 2 illustrates the rhizotron.

Step 1. Remove the protective covering of paper from one of the plexiglass sheets (a). Use a marker to equally divide a full sheet of plexiglass into six rectangular compartments. The measurements should be 24 in (60.96 cm) wide and 32 in (81.28 cm) long. Once the lines have been drawn, connect opposite points making an (x) in each of the boxes. The (x) determines the point at which to drill the hole that will receive the terminal adapter and nut (b). This piece of plexiglass is the top section of your rhizotron (A_1). *Note*: the 24- x 32-in (60.96- x 81.28-cm) grid system worked best for our studies and is optional depending on your specific needs.

Step 2. After marking the glass, compare your hole-saw kit to the size of the terminal adapters you have purchased. The threads of the terminal adapter should fit just inside the hole-saw bit with little room to spare. Proceed to drill holes through the plexiglass at the marked center of the (x). Depending on the terminal adapters you purchased, you may have to grind, sand, or cut the thread length down on the terminal adapters so they will allow room for the roots to emerge against the bottom piece of plexiglass and spread horizontally through the viewing window when the nut is threaded to the underside. *Note*: when drilling holes in the glass, be sure to lay the glass on a flat wooden surface in order to prevent fracturing the glass and dulling the hole-saw bit.

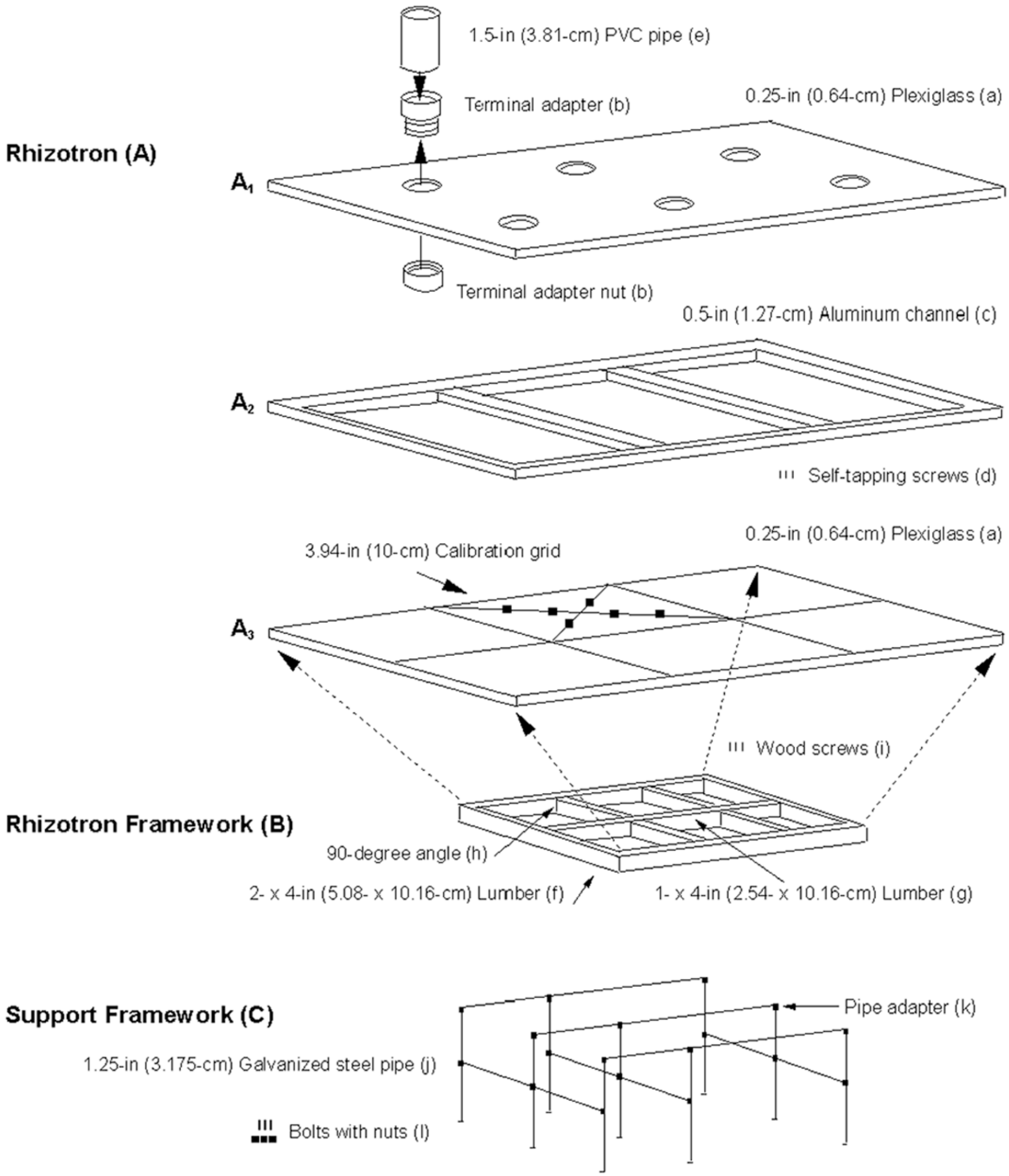
Step 3. After finishing steps 1 and 2, secure the 0.5-in (1.27-cm) aluminum channel (c) to the outside edge of the plexiglass by drilling pilot holes through the plexiglass and the aluminum framework slightly smaller than the self-tapping screws (d).

Step 4. Mark and cut two of the 0.5-in (1.27-cm) aluminum channel pieces that will divide your rhizotron into thirds and connect them to the plexiglass with self tapping screws. These pieces should be 47 in (119.38 cm) long. Use the same procedure as in step 3.

Step 5. Connect the terminal adapters to the top piece of plexiglass making sure the threads are facing the center of the unit. This will be the side the aluminum channel has been attached to.

Step 6. Place the rhizotron on the working surface with the aluminum channel facing up. After removing the protective covering of paper from another full sheet of plexiglass, screw the new sheet of plexiglass to the aluminum channel framework. This should be done in the same fashion as in steps 3 and 4, and will create the bottom of the rhizotron.

Step 7. After the rhizotron is screwed together, for ease of measuring and estimating the root lengths, drill 0.125-in (0.318-cm) holes halfway through the bottom piece of glass every 3.94 in (10 cm). By drilling holes on the angle, or from the center of the planting hole, root length can be measured digitally and estimated when photographing growth due to the pre-established distance that will show up in your digital photograph. In order to establish these holes, draw a grid system as in step 1 so that one can work from the center of the (x) outward. This will increase the accuracy of measurements.



Notes: 1. Drill pilot holes and use self-tapping screws to secure plexiglass to aluminum channel.
2. Use eye screws to secure dark-colored tarps to the rhizotron framework to eliminate light penetration into the underside of the rhizotron.
3. Secure the rhizotron to its framework using wood screws, and place the rhizotron and its framework on the support framework.

Figure 2. *Sketch of an inexpensive rhizotron design, including the rhizotron, its framework, and a support framework. Observations of the root systems are taken on the underside of the rhizotrons. Lowercase letter designations in parentheses correspond to those in the text and table 1.*

Table 1. *List of equipment and materials for construction of an inexpensive rhizotron, along with a rhizotron framework and support framework, used for two-dimensional, horizontal root growth measurements. The designations correspond to those given in the text and figure 2.*

Equipment

Mitre saw, reciprocating saw with blade for cutting metal, screw gun, wrenches, drill bits, hole saw kit, marker

Materials

System component	*Designation*	*Quantity*	*Description of part(s)*
A. Rhizotron	a	2 sheets	Plexiglass [4 ft x 8 ft x 0.25 in (1.219 m x 2.438 m x 0.64 cm)]
	b	6 pieces	Terminal adapter with nut [1.5-in (3.81-cm) diameter]
	c	4 pieces	Aluminum channel [8 ft x 0.5 in (2.44 m x 1.27 cm)]
	d	1 box	Self-tapping screws
	e	48 in (121.92 cm)	Polyvinyl chloride pipe (PVC) [1.5-in (3.81-cm) diameter]
B. Rhizotron framework	f	3 pieces	Lumber [2 in x 4 in x 8 ft (5.08 cm x 10.16 cm x 2.44 m)]
	g	3 pieces	Lumber [1 in x 4 in x 8 ft (2.54 cm x 10.16 cm x 2.44 m)]
	h	10 pieces	90-degree angle
	i	1 box	Wood screws
C. Support framework	j	9 pieces	Galvanized steel pipe [8 ft x 1.25 in (2.438 m x 3.175 cm)]
	k	18 pieces	Pipe adapter [1.25 x 1.25 in (3.175 x 3.175 cm)]
	l	18 pieces	Bolt with nut [3 x 0.25 in (7.62 x 0.635 cm)]
D. Filling and planting	m	3 tarps	Dark-colored tarp [4 x 8 ft (1.219 x 2.438 m)]
	n	1 box	Eye screws
			Planting medium
			Planting stock

Step 8. After the two pieces of plexiglass are fastened to the aluminum channel framework, flip the rhizotron over and cut the polyvinyl chloride pipe (PVC) (e) to the desired length based on experimental objectives and plant material used. We cut our PVC to 8 in (20.32 cm) to accommodate the planting medium and offer vertical support for our 8 in (20.32 cm) cuttings. Insert the PVC into the receiving end of the terminal adapters to complete the rhizotron. By cutting the PVC to 8 in (20.32 cm), the bottom of the cutting will be approximately 0.5 in (1.27 cm) from the bottom piece of plexiglass and allow early root development to be viewed soon after planting. *Note*: for easy cleanup and storage do not glue the PVC to the terminal adapters.

Rhizotron Framework Assembly. Figure 2 illustrates the rhizotron framework.

Step 1. Cut two 45-in (114.3-cm) pieces from one piece of the 2- x 4-in (5.08- x 10.16-cm) lumber (f). This will be attached to the full 8-ft (2.44-m)-long pieces of the 2- x 4-in (5.08- x 10.16-cm) lumber by screwing through the 8 ft (2.44 m) length and into the shorter pieces of wood creating the outside of the rhizotron framework.

Step 2. Cut one piece of the 1- x 4-in (2.54- x 10.16-cm) lumber (g) to 93 in (236.22 cm) and attach it to the center of the 2- x 4-in (5.08- x 10.16-cm) framework by using two of the 90 degree angles (h) and wood screws (i).

Step 3. Cut the remaining pieces of 1- x 4-in (2.54- x 10.16-cm) lumber to 22.125 in (56.197 cm) and install with 90 degree angles and screws. You will end up with four pieces that will fit between the outside framework and the dividing piece of 1- x 4-in (2.54- x 10.16-cm) that was installed in step 2. Be sure to space these four pieces directly under the aluminum framework previously installed in the rhizotron to allow full view of your root-viewing window from below.

Support Framework Assembly. Figure 2 illustrates the support framework.

Step 1. Using a reciprocating saw, cut six pieces of the 1.25-in (3.175-cm) galvanized steel pipe (j) into 4-ft (1.219-m) sections. Divide each 4-ft (1.219-m) section in half by drawing a line with a marker at 2 ft (0.61 m) from either end.

Step 2. Take a section from step 1 to be your horizontal support. Connect the end of the horizontal support at the 2-ft (0.61-cm) line on another of the sections from step 1 using a 1.25- x 1.25-in (3.175- x 3.175-cm) pipe adapter (k). Insert a 3-in long x 0.25-in diameter (7.62- x 0.635-cm) bolt (l) into the adapter and tighten the nut. Repeat this process with another 4-ft (1.219 m) section of steel pipe on the other end of the horizontal support, forming an “H” shape.

Step 3. Connect a third 4-ft (1.219-m) section to the horizontal support at the 2-ft (0.61-cm) line of each section using an adapter and nut as in step 2.

Step 4. Repeat steps 2 and 3 for the remaining 4-ft (1.219-m) sections. *Note*: you should end up with three “vertical units.”

Step 5. Draw a line at 32 in (81.28 cm) from one end of each of the remaining 8-ft (2.438-m) sections of steel pipe. Connect each end of the 8-ft (2.438-m) section to one end of two vertical units using an adapter and nut as in step 2. Similarly, connect the third vertical unit to the 8-ft (2.438-m) section at the 32-in (81.28-cm) line. Repeat the process with the remaining 8-ft (2.438-m) sections of steel pipe. *Note*: do not center the third vertical unit on the 8-ft (2.438-m) sections because the support framework will impede clear view of the middle root-viewing windows.

Securing the System Components. Secure the rhizotron to its framework using wood screws. Place the rhizotron and its framework on the support framework, with the weight of the rhizotron and its framework keeping them in place on the support framework.

Filling and Planting.

Step 1. Our planting medium consisted of one part peat to three parts mason sand. To fill the rhizotron, remove the upper cover and fill, then reapply after leveling the medium in the bottom section of the rhizotron. Our soil mixture allows for easy root removal and viewing of fine roots while photographing the root system. Also, this mixture offers no confusion between peat and root material and still aids in maintaining some moisture if irrigation systems fail during extended leave.

Step 2. After the rhizotron is filled, wrap the sides with 4- x 8-ft (1.219- x 2.438-m) dark-colored tarps (m) to eliminate the potential of light penetration to the underside of the rhizotron (figure 1). In order to do this, attach eyescrews (n) to the 2- x 4-in (5.08- x 10.16-cm) rhizotron framework in line with the grommet openings and allowing the tarps to come into contact with the floor.

Step 3. Before planting any material, test all irrigation systems to ensure proper operation and water volume needed to support the plant material. These volumes are subject to change as plant material matures. *Note*: drainage may be necessary.

Step 4. Plant the cuttings (or other planting stock) in the soil of the PVC (figure 3). *Note*: the size of the PVC depends on the planting stock used.

Rhizotron Data Collection

We developed our rhizotron because existing systems for studying root growth failed to meet our research needs. Our system supports two-dimensional, horizontal root growth measurements over time without disturbing aboveground plant growth and without the need for destructive sampling of roots. Our third objective in the development of the rhizotron was to acquire novel rooting data that can be input to a plant growth model. The uniqueness of the data that can be acquired with this system is a result of the potential to view an entire root system as it develops and comes into contact with root systems of neighboring plants. Therefore, one can map the growth of the roots continuously during development to better understand how root systems respond to contact with obstructions, moisture and temperature gradients, and competition from other plants.

Rooting parameters that can be observed with our system include but are not limited to length and number of primary, secondary, and tertiary roots, as well as parameters associated with the spatial orientation of the roots (root geometry) (figure 4). For example, one can measure the angle of secondary/tertiary root branching over time to observe how roots occupy spaces in the soil. Points of differentiation of lower-order roots from higher-order roots also can be studied. The distance between secondary roots or between secondary roots and the root tip is an example of root geometry data that can be very useful in the development of a computer-generated rooting model. Continual observation of the root system also can lead to the identification and selection of specific genotypes adapted to variable soil conditions. For example, the development of a root system mostly composed of long, thick primary roots compared with one having more secondary and tertiary roots may depend on the genotype.

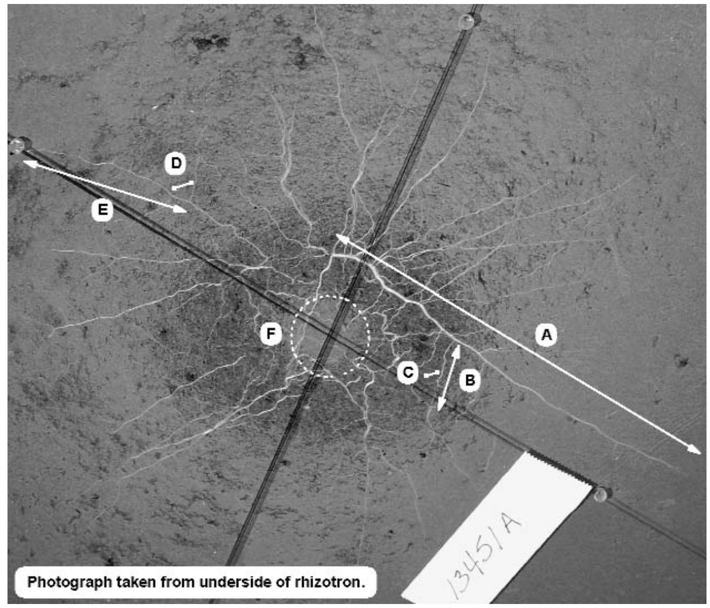


Figure 4. *Root system of a Populus cutting 46 days after planting. Measurements can be taken from primary roots (A), secondary roots (B), and tertiary roots (C), with examples of root geometry parameters such as angle of secondary/tertiary root branching (D) and distance between secondary roots and the root tip (E). The bottom of the cutting is located 0.5 in (1.27 cm) above the dashed, white circle (F). Photographs such as this can be taken throughout development of the root system without destructive sampling.*

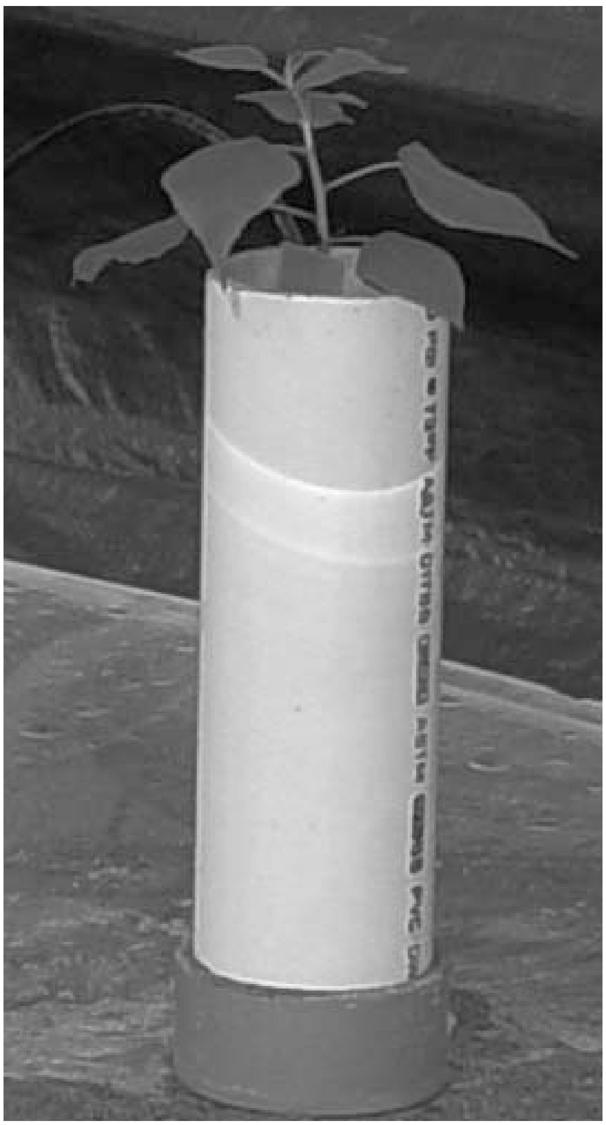


Figure 3. *Populus cutting, 8 in (20.32 cm) long, in a PVC planting tube with a soil medium of three parts sand to one part peat. The photograph was taken 29 days after planting.*

Our rhizotron can be used for more than learning about the structure and development of root systems. For example, the rhizotron can be used to study the effects of simulated browse on the rooting of cuttings or seedlings. In addition, the rhizotron can be used in phytoremediation treatability studies testing if specific genotypes will develop roots in contaminated soil before the genotypes are used for *in situ* trials. The aforementioned use of the rhizotron in competition studies also supports its potential for broad-scale application. We believe, with a balanced combination of scientific and creative ingenuity, our inexpensive rhizotron design can be modified to assist almost anyone interested in learning about plant root systems.

Aknowledgements

We are grateful to Jill Crozier, Rob Doudrick, and Bill Mattson for review of earlier versions of this manuscript.

References

Bohm, W. 1979. Methods of studying root systems. Berlin-Heidelberg: Springer-Verlag. 188 p.

Carlson, C.W. 1965. Problems and techniques in studying plant root systems. In: Proceedings of the 9th International Grassland Congress. [Location of publisher unknown: publisher unknown]: 2: 1491-1493.

Friend, A.L.; Scarascia-Mugnozza, G.; Isebrands, J.G.; and Heilman, P.E. 1991. Quantification of two-year-old hybrid poplar root systems: morphology, biomass, and 14C distribution. Tree Physiology. 8: 109-119.

Gower, S.T.; Reich, P.B.; and Son, Y. 1993. Canopy dynamics and aboveground production of five tree species with different leaf longevities. Tree Physiology. 12: 327-345.

Heilman, P.E.; Ekuan, G.; and Fogle, D.B. 1994. First-order root development from cuttings of Populus trichocarpa % Populus deltoides hybrids. Tree Physiology. 14: 911-920.

Kaspar, T.C.; Ewing, R.P. 1997. ROOTEDGE: Software for measuring root length from desktop scanner images. Agronomy Journal. 89: 932-940.

Kokko, E.G.; Volkmar, K.M.; Gowen, B.E.; and Entz, T. 1993. Determination of total root surface area in soil core

Lauenroth, W.K.; Whitman, W.C. 1971. A rapid method for washing roots. Journal of Range Management. 24: 308-309.

Newman, E.I. 1966. A method of estimating the total length of root in a sample. Journal of Applied Ecology. 3: 139-145.

Orlovic, S.; Guzina, V.; Krstic, B.; and Merkulov, L. 1998. Genetic variability in anatomical, physiological, and growth characteristics of hybrid poplar (*Populus euramericana* Dode (Guinier)) and eastern cottonwood (*Populus deltoides* Bartr.) clones. Silvae Genetica. 47: 183-190.

Stoermer, E.F. 1996. A simple, but useful, application of image analysis. Journal of Paleolimnology. 15: 111-113.

Yorke, J.S. 1968. A review of techniques for studying root systems. St. John's, Newfoundland Forest Research Laboratory Information Report N-X-20. 29 p.

Wu, R.L.; Stettler, R.F. 1994. Quantitative genetics of growth and development in Populus. I. A. three-generation comparison of tree architecture during the first two years of growth. Theoretical and Applied Genetics. 89: 1046-1054.