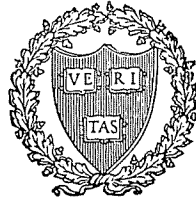


CONTROLLED GROWTH OF
FOREST TREE ROOTS:
TECHNIQUE AND APPLICATION

By

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TECHNIQUE AND APPLICATION

Walter H. Lyford and Brayton F. Wilson¹

ABSTRACT

When woody roots of some forest trees (*Acer rubrum* L., *Quercus rubra* L., *Pinus strobus* L., *Populus tremuloides* Michx.) are severed new roots grow out near the cut surface on the portion toward the stem if this portion is kept continually moist. These new roots, still attached to the trees, can be grown in shallow trays using soil or other growth media and can be manipulated under controlled environment for physiological studies. The technique, a combination of the well known air-layering and root-pruning techniques, is simple, inexpensive and well suited to the study of growth and physiology of roots from mature trees.

INTRODUCTION

Growth and physiology of tree roots have been studied by many methods, among which are the culture of excised roots in nutrient solution (Torrey 1964), observation of roots through glass walls in underground buildings (Rogers and Head 1962, Lyr and Hoffmann 1964), in pots (Leibundgut and Dafis 1964), examination after removal of the overburden of soil (Ladefoged 1939), and by study of roots taken from random soil samples (Kramer and Bullock 1966).

Another method is to produce new root tips at specified places on existing woody roots by a modified air-layering technique, adapting a process that occurs commonly under natural conditions (Lyford & Wilson 1964). This method and a few of its many applications are presented in this paper.

MATERIALS AND METHODS

The modified air-layering technique for growing roots at specified places was developed using red maple (*Acer rubrum* L.). Therefore, most of the experimental data deal with red maple roots, but the technique has been used successfully also on red oak (*Quercus rubra* L.), white pine (*Pinus strobus* L.); trembling aspen (*Populus tremuloides* Michx), and doubtless could be used on most tree species. The basic steps are outlined below. The technique has been used successfully at the Harvard Forest whenever tried, from late May to mid-August.

(1) A woody root is exposed in the soil (Figure 1). This may be a single root traced from the stem, a root chosen at random in an area of the forest floor, or a root exposed by digging a trench. Roots 5-10 mm in diameter are most easily handled.

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Funds for this publication have been supplied from generous gifts by the Friends of the Harvard Forest.

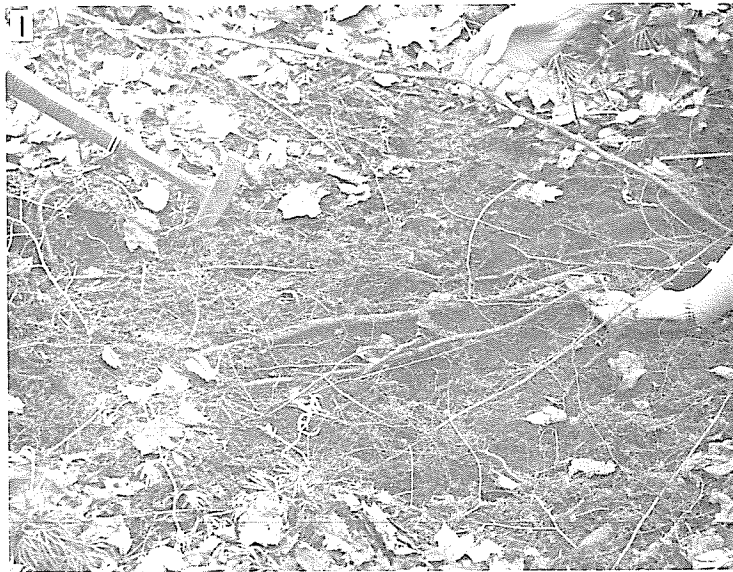


Figure 1 Horizontal red maple roots are readily exposed using simple tools. The roots should be freed for 1-2 m.



Figure 2 After exposure the roots are severed, the ends attached to the tree are encased in moist soil, and the soil is wrapped with plastic sheeting or aluminum foil.

(2) The root is severed. A single root may be cut at any point; roots chopped off in digging the wall of a trench are usually cut again to obtain a clean end.

(3) It is advisable to loosen the root from the soil and from other entangling roots for 1-2 meters from the cut end toward the stem, so that the root is easily moved during the next steps.

(4) The cut end of the root attached to the stem and that portion back from the cut end for a distance of 10-20 cm is wrapped in moist soil. The soil, in turn, is wrapped in aluminum foil or plastic sheeting to prevent drying and to keep the soil firm against the root (Fig. 2).

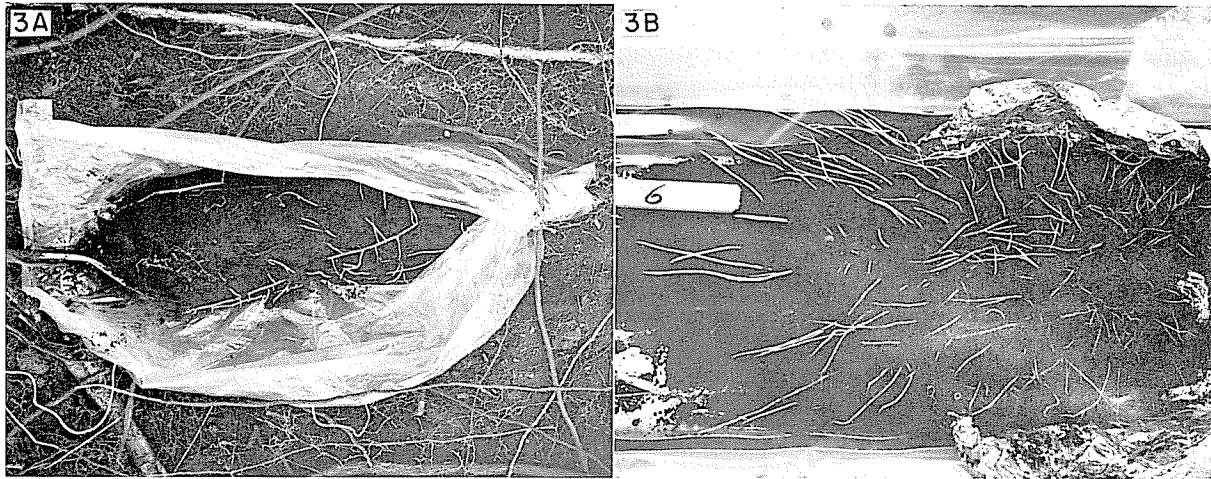


Figure 3 Within 2-3 weeks new roots are clearly visible in the encasing soil.

(5) New root tips 1-3 mm in diameter develop from the woody root and are visible on the surface of the moist encasing soil within 2-3 weeks (Fig. 3). These new roots develop into woody roots (Wilson 1964). Roots severed by merely chopping with an axe through the forest floor, or by other means, and left undisturbed will form new tips on the end attached to the tree if the soil does not dry out within 2-3 weeks. The phenomenon, of course, is the basis for the root pruning technique commonly used by nurserymen. Branching patterns of mature root systems show that this process occurs under natural conditions after wounding by rodents, decay or other natural agencies (Lyford and Wilson, 1964). If the woody root is wrapped locally at some distance back from the cut end (up to 3 meters) new roots also form under the wrapping.

(6) The cut end of the woody root, still wrapped in soil containing the new roots, is placed on a tray or other container lined with plastic sheeting (Fig. 4) and firmly secured so the whole assembly can be moved within limits imposed by the uncovered length of the original root without disturbing the new roots. The wrapping is removed and a 2-5 mm thick layer of fresh soil, sand, or other water-holding medium is spread along the tray. Containers we have found useful include shallow wooden trays varying from 10 to 20 cm wide and 1/2 to 2 meters in length, plywood sheets up to 1 meter wide (to allow spreading of the new roots for individual experimentation), plastic-lined aluminum troughs for nutrient or sand culture studies, or even pots and pails (Fig. 4).

New roots growing out of the sides of trenches can be transferred to trays by loosening the roots back to where they become flexible, but this technique is more troublesome than that just described.

(7) The new roots grow along the tray on or near the surface of the layer of moist soil (Fig. 5). Considerable care must be taken to keep the soil moist enough to avoid drying the roots (sure death), but not so wet as to encourage the growth of decay fungi.

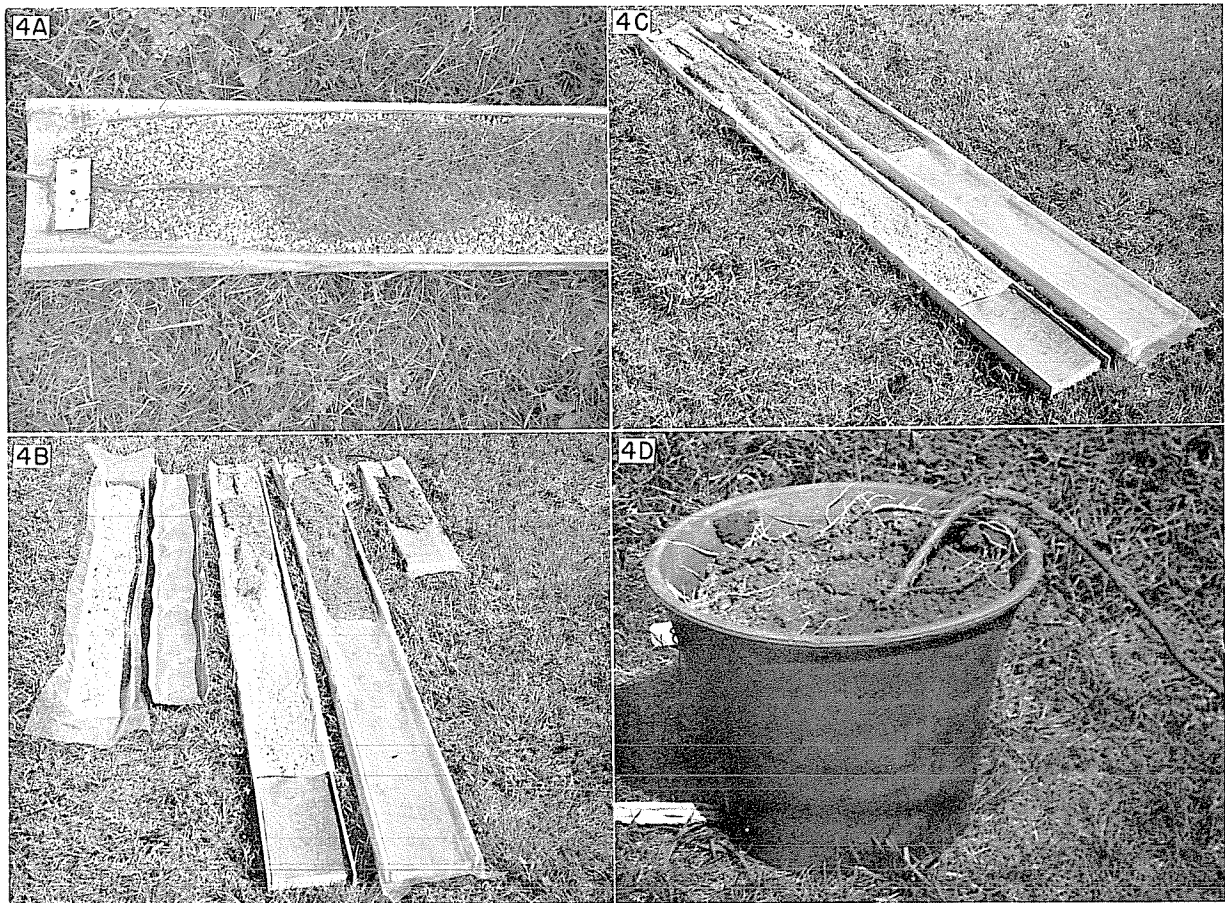


Figure 4 The new roots may be grown in trays, pots, or whatever containers are most suitable for the particular investigation. The parent root should be firmly attached to the container so that the whole root system can be moved without disturbing the growing roots.

In any use of this technique, the major day-to-day problem is watering the roots. If watered by hand, the trays should be checked at least every other day during the summer. Many simple techniques for watering can be devised. A satisfactory arrangement in our work has been to keep moist paper towels both on and under the soil in the trays. Towels distribute the moisture evenly, reducing the possibility of localized drying, and keep the roots at nearly optimum moisture and aeration conditions.

The trays are usually covered with aluminum foil or plastic sheeting to reduce evaporation. Considerably more care to prevent decay is needed under warm than cool conditions.

Water can be supplied semi-automatically by wicks or by drip culture techniques. Strands from ordinary floor mops provide satisfactory wicks. One or more wicks can be used per tray depending on rate of water use (Fig. 6). After a week or two, wicks tend to clog as a result of bacterial growth and have to be replaced. With drip cultures the tray is placed in a sloping position and the water, or nutrient solution, dripped in at the upper end. In our experiment roots growing in sand were watered with Shive's solution, but any other nutrient solution could be used.

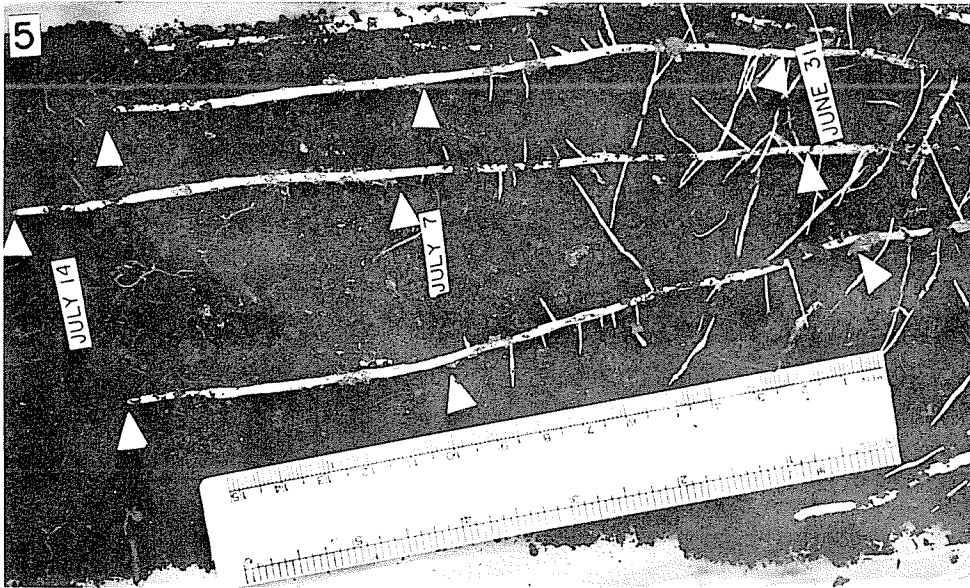


Figure 5 The new roots grow horizontally near the surface of the soil in the trays and have a normal habit. Lateral roots emerge about 10 cm behind the large root tips.



Figure 6 Roots in trays must be kept moist. Wicks made of strands from wet mops provide a simple semi-automatic method of watering.

Once the new roots are growing on trays they are available for observation, measurement, or experiment. For intensive studies it is desirable to build an inexpensive shelter over the trays. We call such a structure a "rhizotron" and the term will be used in this paper. Some desirable features of a rhizotron are; location in the forest itself to allow study of roots attached to mature trees; simple and relatively inexpensive construction; roots raised from the floor for easy working conditions; plenty of head room; electricity

available for lights and instrumentation; and enough room available for movement of long trays (roots grow about a meter per year). Judging from our experience there is a tendency to make the buildings too small. Our first two rhizotrons, about 2 x 4 and 3 x 3 meters respectively, were much too small to enable easy movement of trays, heating units, watering devices and other equipment.

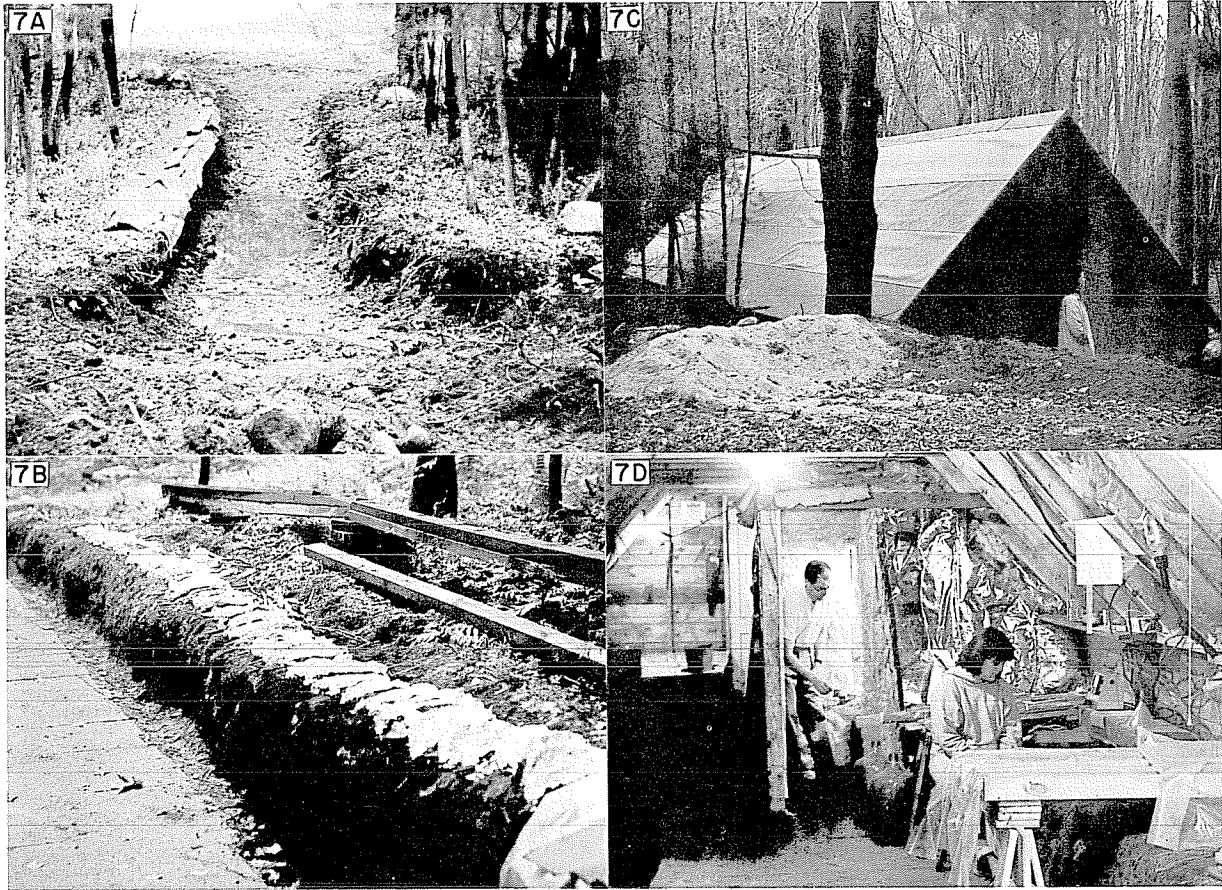


Figure 7 The largest rhizotron at the Harvard Forest was built over a bulldozed ditch (A). New roots are produced along the sides of the ditch (B). The building is about 4 m wide and 16 m long (C), and has ample room inside (D) for trays of roots and insulated rooms used in temperature experiments.

A satisfactory rhizotron at the Harvard Forest, designed for about a three-year life, is shown in Fig. 7. This shed-like building, 4 meters wide and 16 meters long, was built over a 2 meter wide bulldozed trench. The trench, one meter deep, provides roots at bench height on either side. This rhizotron has a simple wood frame and is covered with asphalt roll roofing.

Temperature of roots in trays can be controlled in several ways. At first we used the relatively crude method of placing electrical heating tape under the trays and controlling the heat by a rheostat or built-in thermostat. This method gave uneven heat in different parts of the trays, but was satisfactory for keeping trays warm during the winter. A simpler, more accurate temperature control was obtained by use of electric space heaters with built-in fans and thermostats in small insulated rooms within the rhizotron. Temperature of soil on the trays (measured with a recording thermistor thermometer) was readily maintained at $\pm 1^{\circ}$ C in the individual, insulated rooms.

An important feature of this technique is that roots growing in trays can be handled gently without apparent damage or decrease in growing rate. Red maple roots in trays are remarkably resistant to injury by handling. Although large root tips are stiff and inflexible for 10-20 cm back from the tip, they are flexible beyond that point and can be bent without damage to the root. The stiff portion of the root can be lifted and moved without damage provided lifting is done by touching the root back of the root tip. If the tip of the root is touched and damaged it usually dies, but new lateral replacement roots develop just back of the injury within a week or two.

Roots in our rhizotrons were grown for the most part in the dark, but during observation periods they were exposed to incandescent light. Under conditions used there was no obvious effect of light on roots growth. We have observed that red maple roots can grow in direct daylight without apparent injury, though they do turn red after prolonged illumination.

RESULTS AND DISCUSSION

The growth habit of red maple roots in trays appears to be generally comparable to that described for roots growing naturally in the soil (Lyford and Wilson 1964). The new tips grow rapidly along the tray. About 10-15 cm behind the tips lateral roots emerge and grow out at an angle (Fig. 5). Successive orders of roots are smaller and grow less rapidly. A root system in a tray is necessarily limited to the horizontal plane formed by the layer of soil in the tray. Decay and death of individual root tips is common, as it is under natural conditions, and if large root tips die, replacement tips are formed within two weeks so the root system in the tray continues to enlarge.

Our preliminary observations suggest that red maple root habit is fairly consistent over a broad range of soil texture and fertility if the soil is maintained at near optimum moisture conditions. On the other hand, root habit changes under very moist, poorly aerated conditions, and roots die immediately in local dry areas.

Our most extensive observations have been on the growth of red maple roots and the effect of temperature on their extension rate. In general, the fastest growing root tips were selected for measurement. During the regular growing season these roots commonly grew at rates up to 20 mm/day and occasionally 30 mm/day. The absolute growth rate was related to the diameter of the root (Table 1).

Day-to-day variation in growth rate of red maple root tips was found to be highly sensitive to temperature. Roots in unheated trays showed day-to-day variations in temperature and growth rate that closely paralleled the variation in daily mean outside air temperature (Fig. 8). Roots in trays with controlled temperatures grew at essentially constant rates, independent of outside air temperature. Thus, variability in unheated trays reflected variability in tray temperature rather than outside temperature. In heated trays the rate of elongation is apparently determined by the temperature of the roots themselves and they respond rapidly to changes in temperature (Fig. 9). Although roots grew faster as the temperature was increased (up to 25° C.), they also tended to be subject to more decay at higher temperatures. The optimum temperature for root growth in the trays seemed to be about 12-15° C.

Red maple roots in unheated trays stopped growing about the end of October in 1965, but when heated with electric cables underneath the trays many root tips grew until January 23. Some roots in heated insulated rooms grew throughout the winter of 1965-66 (Fig. 10). During the winter the temperature of the soil outside the rhizotron where the tree stems were located was less than 1° C. Therefore, it was assumed that the low soil temperature

TABLE 1.

Daily elongation of roots of different diameters.

A minimum of 9 roots were measured in each diameter class.

Date	Average elongation in mm	
	Root tips less than 1 mm diameter.	Root tips 1 mm or more in diameter.
September 22	9	19
" 23	13	19
" 24	9	16
" 25	9	16
" 26	9	14
Average	10	17

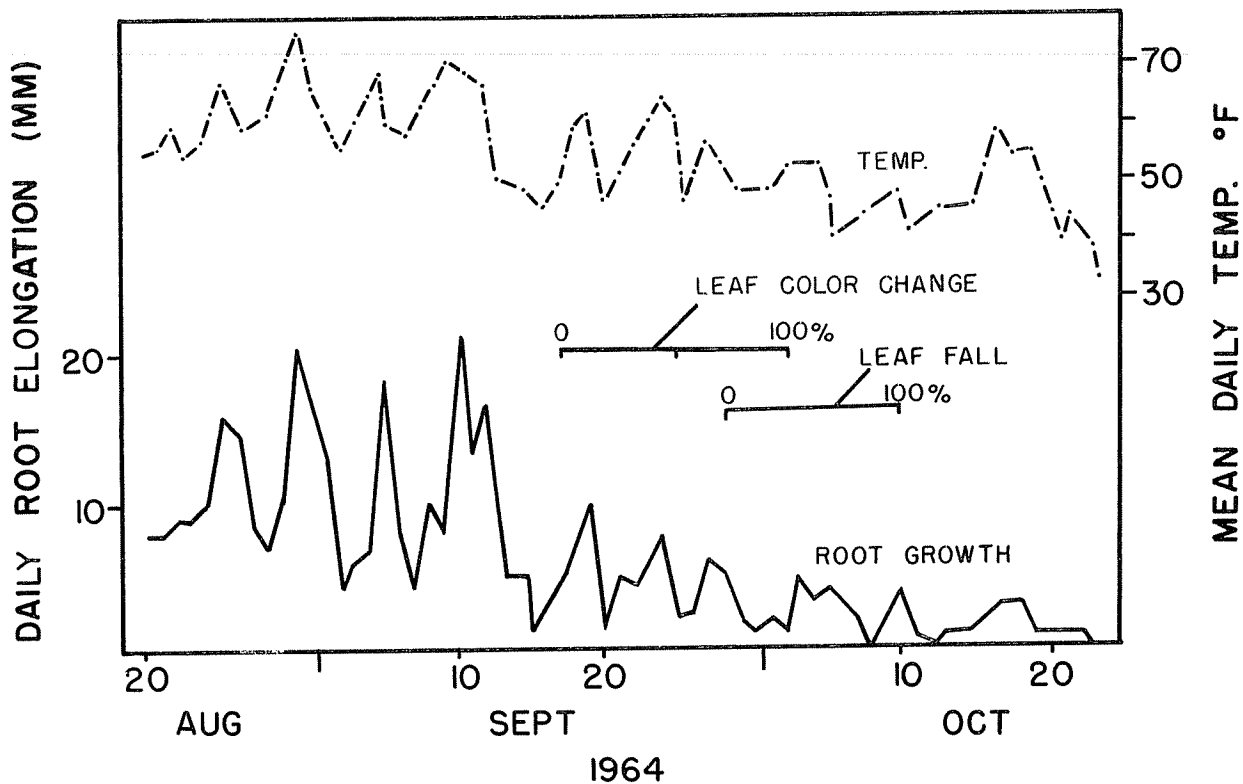


Figure 8 Relationship between rate of elongation of roots growing in unheated trays to the mean daily temperature.

stopped translocation from stems, and the roots grew independently of the rest of the tree. In a test of this assumption, a woody root in January was severed in the tray, so that the tips had no connection to the tree outside the rhizotron. The tips continued to elongate at an undiminished rate for 10 days. The rate of elongation then slowed and stopped, with one tip continuing to grow for 8 more days. These results suggest that there may have been some translocation from outside the rhizotron despite the low soil temperature.

In one tray of red maple roots the tips had stopped growing at the end of October 1964. The tray was heated starting in the middle of November, but the roots grew only for an 8-10 day period during a thaw in December and then grew again in April, about the same time as did unheated roots. Possibly these roots had become dormant and the dormancy was broken during the thaw, but it is difficult to explain why once started they did not continue to grow as did the other heated roots.

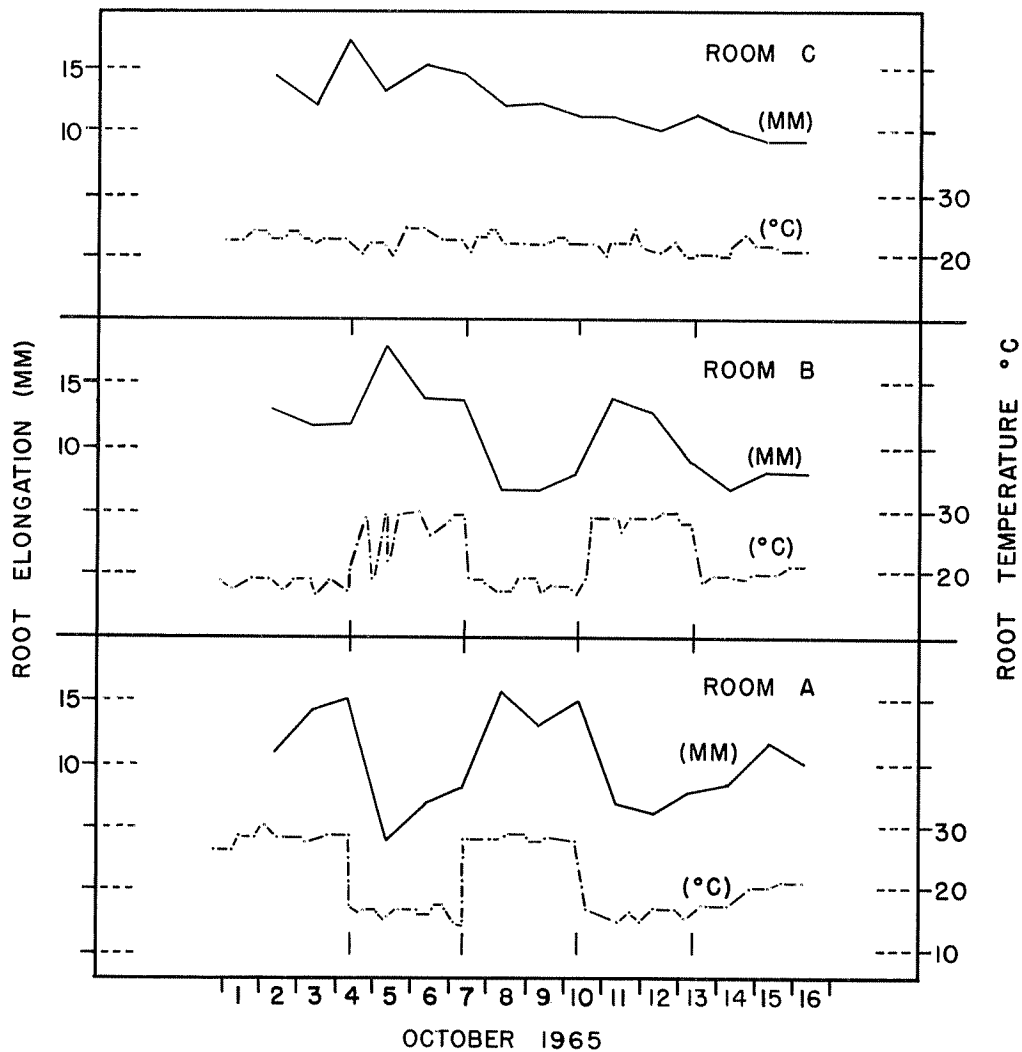


Figure 9 Effect of change in temperature on rate of elongation of roots in trays (average daily elongation of the three fastest growing tips). Roots in rooms A and B were alternately warmed and cooled for 3-day intervals, whereas roots in room C were kept at a constant temperature during the entire period.

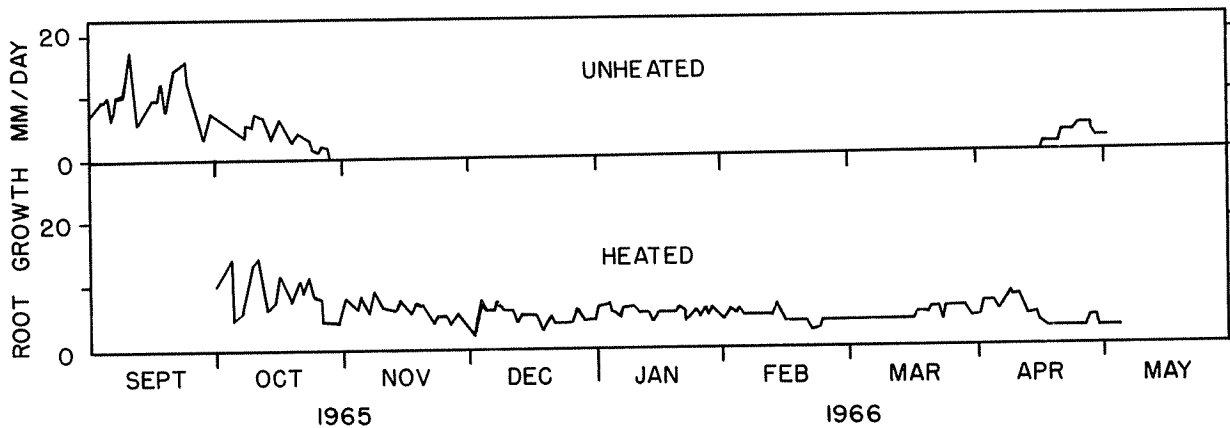


Figure 10 Root elongation in heated and unheated trays. Roots in heated trays were kept at about 20° C starting October 14.

Thus, the technique offers a new approach to the problem of the extent to which root growth is independent of stem control, a problem which has been approached with many other techniques (e.g., Torrey, 1964). Winter root heating experiments showed clearly that roots with limited physiological connection to the stem can grow for long periods and that roots with no physical connection to the stem can grow for shorter periods. Summer experiments showed that the rate of elongation was related to the temperature of the root tips themselves, not to the temperature of the stem.

Some observations were also made on use of water by the root systems growing in the wick-fed trays. Day-to-day variations in water use closely paralleled variation in the rate of evaporation outside the rhizotron (Fig. 11). Water loss from the trays dropped dramatically in early September as the leaves changed color, but paralleled evaporation until early October when about 50 percent of the leaves had fallen. Water loss stayed higher than could be accounted for by evaporation from the trays until about November 1. This loss may have been due to gradual readjustment of moisture in the tree-soil system or to water loss through the bark.

Root tips can be moved or re-oriented without damage to grow in a desired direction or to place them in a new environment. This aspect of the technique was utilized in a study of root growth around barriers. Root tips were placed in holders so that they grew against and then around barriers. Once around the barrier, the root tips tended to orient back to their previous direction of growth (Fig. 12). These results are presented in detail elsewhere (Wilson, 1966).

Advantages of the technique described in this paper are that tree roots may be produced at will by using a modification of a naturally occurring process, and these roots seem to grow at a normal rate and with a normal habit. Once produced, such roots can be grown in a controlled environment and can be manipulated for experimental purposes. Some applications of the technique have been described; there are, of course, many others. Perhaps the distinguishing feature of this technique is that it permits inexpensive, intensive, investigation of the root system of mature forest trees.

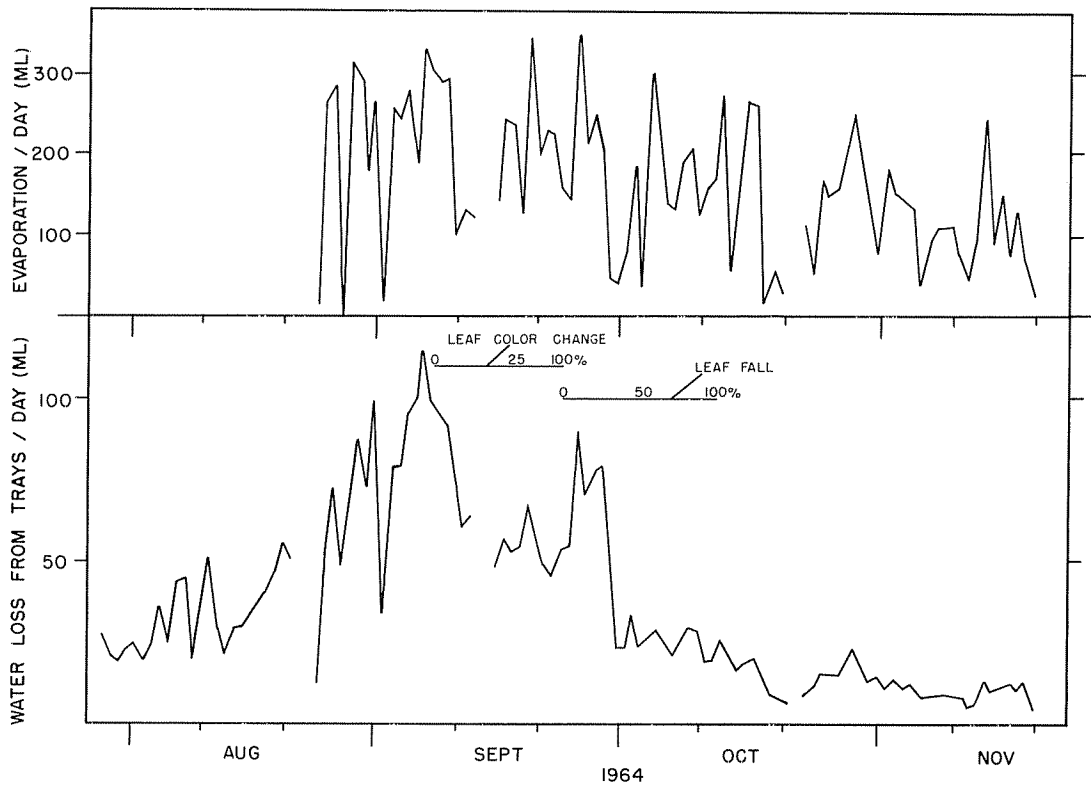


Figure 11 Daily water loss from trays with roots compared with rate of evaporation from a continually moist surface.

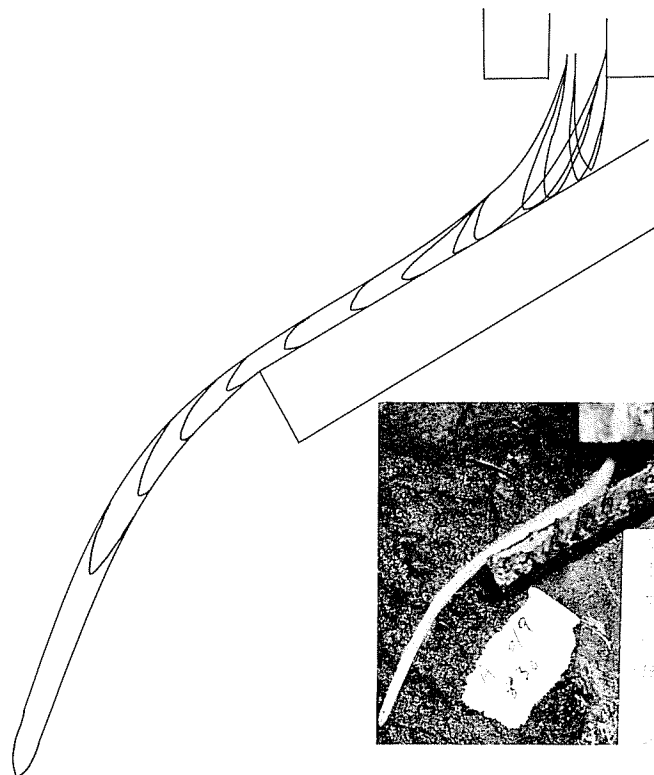


Figure 12 Roots that grow into an angled barrier recurve when they reach the end of the barrier. The drawing is a series of tracings from photographs that show the course of growth around a 60° barrier. Photographs were taken twice a day; scale in the photograph is in mm.

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