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Root Development of Conifer Seedlings as Revealed by Radiophosphorus Absorption and Visual Observation[1]

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The vigor and even survival of planted tree seedlings are to a notable extent dependent on factors relating to root development. The dearth of information regarding such development may be attributed to the difficulties involved in studying roots (Kramer & Kozlowski, 1960, p. 53). The successful use of radio tracers for root investigations of agricultural crops (Hall et al, 1953) suggests that this method may hold real promise in forestry. The technique basically involves injection of a radioistope solution at specific locations in the soil surrounding the roots of seedlings, with periodic analysis of foliage following. The presence of the isotope in the foliage is taken as evidence that at least one root of the particular seedling has reached an injection site, and the amount of radioactivity recorded presents an indication of the activity of the roots in the particular soil zone involved in the injections.

Field studies utilizing the radio-tracer technique have been in progress at Idaho for several years. Results of this work will be published elsewhere. The current report is concerned with a greenhouse experiment carried out in conjunction with the field investigations. The use of glass front boxes as soil containers permitted visual observations of root elongation to be made concurrently with the radio-tracer determinations.

Procedure

The root observation boxes, 2 inches thick, 10 inches wide, and 30 inches deep, were of wood construction with one glass face. Drainage holes were provided at the bottom of each box. The boxes were housed in a wooden frame structure covered with plastic to exclude light from the roots. Supports within the housing allowed the individual root boxes to be angled forward so that roots would tend to grow against the glass, thus aiding observation.

Early in January, 1965, the boxes were filled with a forest soil, which was allowed to settle for several days after moistening. Then the boxes were laid in a horizontal position, the glass fronts removed, and two seedlings per box were placed with their roots flat against the soil surface and root collars even with the soil surface. Immediate replacement of the glass and subsequent watering permitted rapid growth inception. During the experiment, approximately 1 inch of water was applied per week; a thin layer of vermiculite on the soil surface prevented puddling during watering.

The three species used in the experiment were 2-1 Douglas fir *(Pseudotsuga menziesii* (Mirb.) *Franco.)*, 2-1 Ponderosa pine *(Pinus ponderosa* Douglas), and 3-1 Engelmann spruce *(Picea engelmannii* Parry). Ponderosa pine roots were pruned to 9 inches; the others to 8 inches. Sixteen specimens of each species were planted, thus a total of 24 observation boxes were required.

On January 11, the glass was removed briefly to inject phosphorus 32, used as the radio-tracer. The solution employed contained 15 uc/ml of radioactivity. Ten 5-ml injections were made in a horizontal band across the root box. Three placements (treatments) were involved, with injection bands 2, 6, or 10 inches below the root tips. Treatments were replicated four times on each species. Soil in boxes supporting four seedlings of each species was left uninjected to help detect

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possible physiological or morphological abnormalities induced by the radioactivity.

Root-growth records of all specimens, as observed through the glass fronts of the boxes, were maintained in conjunction with records concerning radioactivity.

Twenty to 30 mg of needles were taken for radioactivity analysis from leaders and laterals of each seeding at intervals of 1 week until visual observation indicated that the roots approached the radioactive zone. Then samples were taken three times weekly until the radioactivity appeared in the tissue. These needles were ashed according to standard procedures and analyzed for radioactivity with a Nuclear-Chicago gas flow detecter system. Any significant time differential between root contact (as visually noted) and radiophosphorus translocation to foliage should have been recorded by this method. The data were subjected to an analysis of variance to investigate possible interactions between species and depth of isotope placement zone and isotope detection in foliage. Measurements of the radioactivity of foliage were made periodically throughout the experimental period. The total phosphorus content of the seedlings was obtained at the conclusion of the study.

One Douglas fir and one Engelmann spruce seedling were pressed and subjected to radiographic analysis at the conclusion of the experiment. These autoradiagrams were used to examine areas of high P^{32} concentration in the plants and to detect possible differences in distribution patterns of the isotope between the two species. Kodak No-Screen Medical X-ray Film was used for the autoradiograms. Developing procedures utilized were specified by the manufacturer.

Results and Discussion

Data concerning root growth rates of the three species are presented in Table 1. While four specimens were initially utilized per treatment, one plant from each of two treatments died soon after the work began. In a third instance, visible roots of one specimen never did reach the injection band. For these particular treatments, averages are based on three plants, as indicated in the table.

Growth inception, while slow due to the initial dormant state of the plants, was actually not retarded as much as these particular data might indicate. Measurements are based on distance from the lowest of the pruned root tips to the particular injection band. Most new growth, however, originated in the upper root system and not at the pruned tip. Thus, while the most rapidly growing specimen required 30 days to penetrate 2 inches below the pruned root tips, this normally required 4 to 8 inches of new root growth. Douglas fir and Engelmann spruce seedlings averaged 30 days for visible roots to reach the 2 inch line, and ponderosa pine was slower, requiring an average of 35 days.

Table 1. Rates of root-growth in root boxes. Number of days required from planting in root boxes until visible new roots penetrated to indicated Depth below the initially-pruned root-tips.

Species	Depth penetrated (inches)	Days required for penetration Minimum Time	Average Time
Douglas-fir	2	30	30
	6	42	56.7[2]
	10	70	72.3[2]
Ponderosa pine	2	30	35.3
	6	32	42.7
	10	50	55.3[3]
Engelmann spruce	2	30	30
	6	35	54.7
	10	58	81.5

[1]Unless otherwise noted, averages based on four specimens.

[2]Average based on three specimens only because of mortality.

[3]Average based on three specimens only because no visible root on one seedling reached injection band during experimental period.

The ponderosa pine roots reached the 6- and 10-inch depths more rapidly than did the roots of either of the other 2 species. For example, ponderosa pine required an average of only 55.3 days for visible roots to penetrate to the 10-inch line, while the Engelmann spruce averaged 81.5 days to reach this point. However, the fastest growing spruce required 58 days, or only 8 days more than the first ponderosa pine to reach the 10-inch depth. This erratic performance within a species can be accounted for by two reasons:

1. Only roots growing against the glass could be measured. Some roots hidden in the soil may have reached an injection zone before the visible roots. Thus, the average time for a particular species to reach an injection band as determined by this method may be somewhat greater than it was in actuality. As will be noted below, in some instances P^{32} uptake was detected before roots were observed to enter an injection band. This is further evidence that some unseen roots elongated more rapidly than those against the glass.

2. A second reason for differences in growth rate observed within species has to do with inherent genetic variation. More replication than was possible in this study would help alleviate the effect of this factor.

Root growth increments were variable from week to week (Table 2). Average growth for all seedlings ranged from 0.90 inches per week to 2.5 inches per week over a 7-week period. The analysis of variance indicated that ponderosa pine roots grew significantly faster than either of the other species. Only data from February 8 through March 22 were used to test for significance, because several ponderosa pine roots had reached the bottom of root boxes by that time. Later data were biased as further measurements of these roots were inaccurate.

Table 2. **Weekly root-growth increments of seedlings in root boxes, greenhouse, 1965. Results are presented in inches.**

Dates		Douglas-fir	Ponderosa Pine	Engelmann Spruce	Average
February	3	2.6	1.3	2.2	2.0
	8	1.8	2.1	2.2	2.0
	15	0.7	2.0	0.7	1.1
	22	0.3	2.4	0.6	1.1
March	1	0.6	1.8	0.3	0.9
	8	1.4	3.1	0.5	1.7
	15	2.7	3.7	1.0	2.5
	22	2.0	1.8	0.9	1.6
	29	1.9	0.9[1]	0.8	
April	5	1.8	0.7[1]	1.5	
	12	1.7	0.2[1]	1.7	

[1]Accurate measurement impossible because some seedlings had reached bottom of root boxes.

Detectable amounts of radioactivity in needle tissue were usually found within a week of the time roots of seedlings were observed to enter a zone of isotope placement (Table 3). Twelve seedlings (over 1/3 of those which lived through the experimental period) had radioactive foliage on a sampling date within a day or two of the time visible roots entered a P^{32} injection band. For the purposes of this investigation, detection within this short time span was considered essentially immediate, and time differential between observation of roots in the isotope placement zone and detection in the needles of the 12 specimens is recorded as 0. Another 13 seedlings developed radioactive foliage either a week prior to or a week after visible roots entered the placement zone. Time differentials exceeded 2 weeks in only five instances. One Engelmann spruce seedling had radioactive foliage 6 weeks before roots were seen entering the isotope placement zone involved, and one ponderosa pine developed radioactivity, although no visible root entered the injection band. This situation can probably be explained by rapidly elongating roots hidden in the soil mass reaching the zone well ahead of the visible roots, and extracting detectable amounts of the isotope almost immediately. Excavation of root systems at the conclusion of the experiment revealed that the ponderosa pine seedling mentioned above, did, indeed, have roots within the soil mass which had penetrated the injection zone.

Table 3. **Time differentials between first observations of roots entering isotope injection zone of soil in root box and detection of radio-activity in foliage of seedling's crown. Instances where there was a lag of a week or more before radioactivity detection are indicated by (+), and where radioactivity was detected before roots were observed to enter a placement zone, by (—). (0) indicates almost immediate detection. Values represent weeks.**

Depth of isotope placement zone	Douglas-fir	Ponderosa Pine	Engelmann spruce
2 inches	+1	+1	+1
	+1	0	0
	+2	+1	+1
	0	0	+3
6 inches	0	+1	-6
	-1	0	+2
	dead	+1	+2
	-2	-2	0
10 inches	+3	0	-1
	-1	+3	+1
	dead	0	-1
	0	[1]	-2

[1]Visible root did not enter injection zone during experimental period.

Three seedlings had roots which were seen to enter the isotope placement zone considerably before needles became radioactive. Apparently absorption of isotope by these particular roots was low, and minimal quantities necessary for detection were slow in accumulating.

An analysis of variance indicated that the three species involved behaved similarly in respect to time differentials observed, and these differentials were not affected by depth of the particular zone of soil radioactivity.

Radioactivity analysis of needle tissue from all seedlings were periodically conducted from the time roots were first entering the isotope placement zones until the end of the experiment. Data concerning radioactivity of foliage sampled (in each instance 1 month after the first detection of the isotope in the particular specimen) are presented in Table 4. Values are presented as activity (expressed in counts per minute) per 10 mg of oven-dry tissue. In order to make the information from all specimens comparable, decay of the isotope was taken into account. Thus cpm values were adjusted to indicate the level of radioactivity which would be present in the tissue had no decay taken place since placement of the isotope.

The tabulated data reveal large variations in radioactivity present in the needles of individual specimens 1 month after first contact with the isotope. Radioactivity was not detected at all in several plants, although it had been present initially. Evidently these particular seedlings absorbed little additional P^{32} during the month interval, and the P^{32} originally present had decayed below a detectable level.

In spite of the variation encountered, several trends appear to be evident. This is especially true considering data involving only the seedlings

Table 4. **Radioactivity 1 month after original uptake in the root-box experiment, 1965. Values in cpm per 10 mg of needle tissue.**

Species	Treatment Depth	Tree number 1	2	3	4
Douglas-fir	2 inches	845	1235	750	825
Ponderosa pine		204	72	237	634
Engelmann spruce		1180	72	0	0
Douglas-fir	6 inches	0	0	dead	519
Ponderosa pine		476	279	281	872
Engelmann spruce		487	0	144	333
Douglas-fir	10 inches	0	0	dead	0
Ponderosa pine		40	167	0	0
Engelmann Spruce		[1]	[1]	200	0

[1]Data not available because experiment was terminated 1 week after uptake was first detected in these specimens.

of each species which showed the greatest amount of radioactivity within each of the depths of isotope placement treatments.

For Douglas fir and Engelmann spruce, the highest levels of radioactivity were detected in foliage from specimens growing in root boxes which had received isotope injections at the 2-inch depth. The most radioactivity detected in a ponderosa pine seedling, on the other hand, occurred where injection was made at the 6-inch depth. Generally, more active absorption of P^{32} occurred in roots situated in either the 2- or 6-inch depth placement zones than in roots growing in the 10-inch depth band.For example, 1 month after foliage radioactivity had first been detected in each specimen, the total activity of needle samples from the 4 ponderosa pines involved in the 2-inch depth treatment was 1147 cpm (based on 10 mg oven-dry weight per sample and adjusted for decay.) The comparable value for the 6-inch depth of isotope placement treatment was 1908 cpm. In contrast, a total activity of only 207 cpm was detected in the samples of ponderosa pine foliage collected from specimens growing in soil injected at 10 inches (2 of the seedlings from this treatment had no detectable radioactivity.)

Total phosphorus content of these seedlings was obtained at the conclusion of the experiment. Values ranged from as low as 1320 ppm up to 4280 ppm in oven-dried tissue. The ponderosa pine seedlings showed a lower total phosphorus content than either of the other species, ranging from 1440 to 1790 ppm. Engelmann spruce seedlings contained an intermediate amount of phosphorus, and the Douglas-fir seedlings contained considerably higher amounts, ranging from 3290 to 4280 ppm. The amount of phosphorus added in the form of P^{32} was negligible. Only 750 uc, amounting to only 2.63×10^{-10} grams of phosphorus, was applied to each root box (except controls.)

Autoradiograms were made from one Douglas fir and one Engelmann spruce seedling to locate areas of high P^{32} concentration. The radioactivity in both cases was very high in roots. In the foliage it was concentrated in all areas of meristematic tissue. Older needles and stems contained considerably smaller amounts of the isotope, as indicated by the lower intensity of images in developed film. No distributional differences related to species were noted.

LITERATURE CITED

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