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Root distribution of forest understory species determined with I-131

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ROOT DISTRIBUTION OF FOREST UNDERSTORY SPECIES DETERMINED WITH I-131

By

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INTRODUCTION

Plant growth is dependent upon the soil as a medium for rooting. Since edaphic factors such as moisture and temperature influence the development of plant roots, they may also influence plant growth.

The ecologist frequently measures soil moisture as a part of characterizing the edaphic factors important to plant growth, but often the depths for collecting soil moisture data are not known. These data should be obtained for those depths at which plant roots are abundant, since the portions of the soil profile in which rooting is profuse are most important in supplying the plant with moisture. Therefore, a knowledge of plant root distribution is necessary for making meaningful soil moisture measurements. One objective of this study is to characterize plant root distribution in order to assess the validity of measuring soil moisture to predetermined depths at the site of investigation.

Root distribution has been studied primarily by mechanical excavations which are both time consuming and laborious and necessitate disturbing the site. The radiotracer technique largely overcomes many of these objections and has been used successfully in many root investigations. The second objective of this study is to investigate the use of the radiotracer technique in fine-textured soils and in stony soils.

REVIEW OF LITERATURE

The distribution of roots in the soil has been studied by four general methods: (1) mechanical excavations of the soil and plant root systems, (2) soil moisture depletion, (3) rare ion injection methods, and (4) radioisotope methods.

Mechanical methods

A common method of studying root systems is by digging a trench and directly observing the roots (Weaver 1915, 1919, 1920, 1922 and Weaver and Jean 1924). Pavlychenko (1937) describes a method of excavating a soil block containing the whole root system of a plant. The soil is washed off with a spray of water and the entire root system is suspended in an illuminated tank for characterization and photography.

A hydraulic root excavation technique was used by Stoeckler and Kluender (1938). A pit six to ten feet deep was dug close to the plant; water was pumped from below the water table and sprayed against the wall of the pit to expose the root system.

The main advantage of mechanical methods is that the root system is directly observed, and many characteristics of the root system such as branching and horizontal extension are readily seen.

Mechanical methods have certain limitations and disadvantages. (1) They are very time consuming and laborious. (2) Even with hydraulic methods, fine roots are often torn from the root system, so maximum root spread is not easily observed. (3) No differentiation between living and dead roots can be made. (4) It is necessary to disturb the site. (5) Water supplies are often limiting for hydraulic excavations. In wild-land

studies a water table is often inaccessible and hauling of water would be very expensive.

Soil moisture depletion methods

Soil moisture depletion methods allow the study of plant root systems in situ. Removal of soil moisture is an indication of the presence of moisture absorbing roots. Doss et al. (1960) and Bennet and Doss (1960) studied the effects of soil moisture levels on root distribution of several forage species. Root distribution was characterized by soil moisture extraction as measured by gypsum blocks. The results of the study indicated that increased soil moisture levels corresponded with a decrease in rooting depth.

Wood (1960) compared rooting depths with soil moisture removal as measured by gypsum blocks. Again, increased soil moisture levels were correlated with decreased rooting depths.

An advantage of soil moisture extraction methods is the fact that moisture absorbing roots only are measured. Visual observations of roots do not differentiate between active and inactive roots.

The disadvantages of relating soil moisture depletion and rooting are: (1) Precipitation during the study period, as well as upward capillary movement of water, make interpretations of soil moisture difficult. (2) Rooting depths of individual species cannot be characterized since depleted moisture patterns are the result of absorption by all the vegetation on the site. (3) The site must be disturbed in order to place many of the soil measuring devices.

Rare ion method

An ion, such as lithium, which is not present in abundance in

the soil and yet it is taken up by plant roots may be injected into the soil profile at various depths (Sayre and Morris 1940). After sufficient time for translocation of the ion to the leaves, plant tissue samples may be taken into the laboratory and analyzed for the element. This method allows in situ study of root systems, but depends on the mobility of the ion outward from the injection point. This can be a problem on soils with high cation exchange capacity. Low mobility of the ion from the injection point reduces the chance of roots picking up the ion.

Radioisotope methods

A radioisotope technique for studying root systems was first developed by Hall et al. (1953) in an investigation of corn, tobacco, cotton, and peanut root systems. Phosphorus-32 was injected into the soil at various depths and radii from the plant, and radioactivity measured in tissue samples taken at several intervals following injection. This technique was later adapted to a study of root systems of southern grasses (Burton et al. 1954) and alfalfa (Lipps et al. 1957). Information on proper fertilizer placement has been obtained (Hammes and Bartz 1963) for various vegetable crops by the radiotracer method. Isotopic tracers were used similarly in studying the uptake of subsoil nutrients by corn roots (Murdock 1955, Murdock and Engelbert 1958, and Waugh 1963). Phosphorus-32 was used by McClure and Harvey (1962) and Nakayama and Van Bavel (1963) to measure root growth and activity in sorghum. Alfalfa root activity has been characterized using radioactive tracers (Lipps et al. 1957 and Lipps and Fox 1964). Root development of artificially seeded plains bristlegrass was studied by injection of

radiophosphorus into the soil (Mathis et al. 1965). A different technique involved injection of P-32 into the stem of wheat plants (Racz et al. 1964). After allowing enough time for translocation of the injected solution, the plant was cut off and soil root cores taken at various depths and their radioactivity measured. Neilson (1964) treated above-ground vegetation with C-14, excavated a monolith of the root system, and made an autoradiograph of the monolith. Unfortunately, these methods involving monoliths necessitate disturbing the site.

All of the above studies center around single agricultural crops rather than natural plant communities. The root systems of grassland communities have been studied with the phosphorus-32 injection technique (Boggie et al. 1958 and Boggie and Knight 1960 and 1962). The study was conducted on four areas with soil textures ranging from sand to a loam with many stones, overlying an indurated layer. Plots were 40 x 40 cm. with five injections per plot. A steel spike was hammered into the soil, removed, and a tube placed in the hole. A ramrod was inserted in the tube to remove soil accumulated in the tube. After removal of the ramrod, 50 ml. of radioactive phosphate solution was poured down the access tube, followed by 50 ml. of water rinse. The sphere of solution spread was over 6 inches in diameter. Results pertinent to the present study are discussed later.

The use of phosphorus-32 has certain limitations. Counting of beta rays requires that the sample be ashed and of uniform geometry to reduce self-absorption of the rays if quantitative measure of radioactivity in the plants is desired. In leached, acid soils, phosphorus is readily fixed by iron and aluminum (Buckman and Brady 1960, p. 438)

and is therefore relatively insoluble and immobile. A certain amount of immobility is necessary so that the isotope placements do not overlap, but it is necessary that the ion move sufficiently from the injection point to contaminate a relatively large (3-6 inch diameter) sphere of soil.

The root systems of trees have also been studied by the use of radioisotopes. Lateral and vertical spread of longleaf pine roots has been determined by injecting iodine-131 into the soil (Ferrill 1963, Hough 1963, McCormack 1963, and Hough, Woods and McCormack 1965). Lateral root extension of longleaf pine (Pinus palustris) approached 51 feet. Lateral root spread was determined for Douglas-fir by injecting rubidium-86 into the bole and later locating the roots with a counter (Staebler and Radiske 1959).

An excellent tracer method was developed by Saiz del Rio et al. (1961) for studying coffee tree root systems. Rubidium-86 was injected into the soil at various depths and radii from the trunk of the trees. Leaf samples were later collected and then radioactivity measured. The study showed that most of the roots were concentrated near the soil surface and close to the trunk.

Price (1965) studied the root pattern of sagebrush in sandy loam soils using iodine-131. The isotope was injected via 3/8-inch aluminum tubes placed at various depths and radii from the sagebrush plants. A scintillation detector was used to measure radioactivity in intact plant tissue. If a plant became radioactive it was assumed that its roots had penetrated to the position of isotope placement. Particular limitations of the method as discussed by Price are: (1) The method is

limited to relatively stone-free soils because of difficulty in tube placement. (2) Iodine can be toxic in large amounts. (3) Soil moisture influences the extent of I-131 movement from the bottom of the injection tube. Movement was less when the soil was dry than when the soil was moist. (4) Due to the short half-life (8.05 days) of I-131, only a limited amount of time for plant uptake can be allowed (about 5 days); if a longer time is allowed, the isotope will be of much lower activity and harder to detect. (5) If any one part of the root system comes into contact with the isotope, all shoots will not necessarily become radioactive as vascular tissues are often continuous from root to shoot on one side of a plant. (6) Because of all the variables, the quantity of I-131 in the shoots is meaningless. The data can only be interpreted on the basis of presence or absence of the isotope in above-ground tissues.

Results of rooting depth studies

Investigations have previously been conducted on several of the species included in the present study. Cox (1959), in a study in ponderosa pine stands on Lubrecht Experimental Forest, investigated root distribution on three different soils. One of these soils was the Lubrecht series, the same soil on which the major part of the present study was conducted. A pit was dug and all roots were counted according to specified size classes. The results on the Lubrecht soil series are shown in Table 1. About 87 percent of the roots were concentrated in the upper two feet of the soil profile. Cox also noted that the subsoil of the Lubrecht series was the poorest medium for root growth of the three soils studied.

Table 1. Root distribution in Lubrecht loam. (From Cox 1959).

Depth (feet)	Number of roots by size (inches)			
	0.1	0.1-0.3	0.3-0.5	0.5-1.0
0-1	2062	113	11	2
1-2	1249	38	7	2
2-3	394	8	----	----
3-4	145	1	----	----

Berndt and Gibbons (1958), using a trench method, studied root distribution of tree and understory species in ponderosa pine types in Colorado. Root systems were characterized on three soils: a gravelly, sandy loam, a stony loam, and a skeletal soil on sandstone. Kinnikinnick (Arctostaphylos uva-ursi) rooting depth was two feet on both the stony loam and the skeletal soil and three feet on the gravelly sandy loam.

Weaver (1919) studied several species by mechanical excavation. Maximum rooting depths for kinnikinnick, snowberry (Symphoricarpos vulgaris) and strawberry (Fragaria virginiana) were 46 inches, 65 inches, and 14 inches, respectively.

Boggie et al. (1958), using the radiophosphorus injection technique, studied root distribution patterns of several native species including yarrow (Achillea millefolium), four species of sedge (Carex), and two species of bedstraw (Galium). Yarrow rooted to approximately 24 inches in sand. Carex arenaria rooted to 40 inches in sand, and the other Carex species rooted to only 4 inches on a stony loam, overlying an indurated layer. Rooting is probably inhibited by the indurated layer. Rooting depth of Galium hercynicum was 24 inches on a sand and

a sandy loam and 12 inches on a stony loam with an indurated layer below. This compact layer appears to have inhibited rooting.

The present study will be conducted on a soil with a heavy textured B horizon. Several investigators have studied root penetration of compact and hardpan layers in soil profiles. Duncan (1941) found the B horizon of a clay soil to inhibit root penetration except where there was a channel or cleavage in the horizon. Penetration by the taproot of Quercus macrocarpa through an indurate stony glacial till was minimal (Crossley 1940). Rooting through a fine-textured soil of blocky structure was along cleavage planes (Diebold 1933), but roots did not penetrate a massive fine sand horizon.

DESCRIPTION OF THE STUDY AREAS

Location

The study was conducted on the Lubrecht Experimental Forest of the School of Forestry, University of Montana. Lubrecht Forest is located 30 miles northeast of Missoula in the Blackfoot River drainage.

The two study areas were located near plots of a soil moisture-ecology investigation. The soil moisture-ecology investigation involves a characterization of soil moisture and temperature, plant and animal phenology, and meteorology for seven sites, designated "ecounits," on a gradient from very dry to very wet. One root study area was located about 100 feet from ecounit 3, a mesic site near the forest camp at an elevation of 4,100 feet. This site is called ecounit 3. The other root study area was located about 300 yards north-northeast of ecounit 5, a moist site near Coloma (elevation 5,900 feet). This site is called ecounit 5.

Climate

Weather data have been collected at the Greenough Post Office near ecounit 3 for several years (Steele 1965). Average annual temperature over a nine year period was 39.2 degrees F. and average annual precipitation for a seven year period was 17.6 inches.

Weather data for ecounit 5 are incomplete. Average daily temperature for January in one year was 23.0 degrees F. and 60.4 degrees F. for July. Total precipitation from June 1 to September 30 in one year was 7.15 inches (R. W. Steele, University of Montana; personal communication 1966).

Vegetation

The study site adjacent to ecounit 3 was located in an opening of a well-stocked stand of ponderosa pine[1] (Pinus ponderosa), Douglas-fir (Pseudotsuga menziesii), and western larch (Larix occidentalis). Understory species consist of low shrubs, grasses, sedges, and forbs, most of which were sampled. A vegetation analysis of the understory is shown in Appendix I. Density data were collected in twenty-four 20 x 50 cm. plots distributed in a regular pattern within the study area.

A dense stand of Douglas-fir and a few lodgepole pine (Pinus contorta) constitutes the overstory near ecounit 5. A great deal of downfall exists at this site. A vegetation analysis of the understory appears in Appendix II. These data were collected in six randomly distributed 20 x 50 cm. plots within the study area.

Geology

Ecounit 3 is located on tertiary basin deposits of poorly consolidated mudstone (Brenner 1964) with a slope of less than 5 percent. The study plots were on a nearly level site of the same material.

Ecounit 5 is located on a long, uniform, north-facing slope of 15 percent gradient. The parent material at this site is sandstone colluvium.

Soils

The soil profile description for the site at ecounit 3 (Table 2) is a composite of the profile descriptions of four separate pits and are described according to Soil Survey Manual, U.S.D.A. 1952. These four

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[1]Nomenclature throughout text from Moss 1959.

descriptions (Appendix III) show the variation in soil physical properties and pH values over the plot area. This soil is a member of the Lubrecht series which is a modal Gray Wooded soil characterized by a leached A2 horizon underlain by a highly illuviated B2t horizon. Textures, as determined by the hydrometer method (Bouyoucos 1936), of the A2 were all silt loams while those of the A2 & B2 varied from a clay loam to a silty clay. The textures of the B2t were silty clay and clay. A loam with various amounts of stratified gravels (0-70%) appeared in the C horizon. The A2 and A2 & B2 horizons had generally moderate subangular blocky structure while the B2t had predominately strong blocky to prismatic structure. Horizon thickness and depth varied somewhat. All of the A2 horizons were 0-5 inches in depth and the A2 & B2 horizons were about 5-12 inches in depth, but the B horizons varied considerably; the upper limit of the B was 10-12 inches from the surface, but the lower limit varied from 33 to 46 inches from the soil surface.

Soil pH values were obtained by the saturated-paste method as outlined by Jackson (1958) and these varied from 5.5 to 6.9 in the solum and were as high as pH 7.4 in the C2 horizon.

A soil profile description for the site near ecounit 5 appears in Table 3. Only one profile was described at this site. The soil is closely related to the Holloway series, a Brown Podzolic soil, which is characterized by a reddish-brown Bir overlying an A2 & B2 horizon, beneath which is a B2t. This particular profile is strong to medium acid throughout and of sandy loam texture in the Bir and A2 & B2 horizons and sandy clay loam in the B2t and C horizons. The main feature of this profile is the presence of a very high (30-60 percent by ocular estimate) percentage of sandstone coarse fragments, generally less than six inches in diameter.

Table 2. Composite soil profile descriptions at ecounit 3

O1 & O2 2 - 0 inches	Forest litter and partially decomposed litter.
A2 0 - 5 inches	Pale brown (10YR 6/3 dry),[1] to dark brown (10YR 3/3 moist) friable silt loam;[2] weak, medium subangular blocky structure; many roots; slightly acid; clear, smooth boundary.
A2 & B2t 5 - 12 inches	Light yellowish brown (10YR 6/4 dry) to dark yellowish brown (10YR 4/4 moist) slightly firm clay loam; moderate to strong subangular blocky structure; many roots; medium acid; abrupt, irregular boundary.
B2t 12 - 33 inches	Light yellowish brown (10YR 6/4 dry) to dark yellowish brown (10YR 4/4 moist) firm clay; medium, strong, coarse prismatic to medium, strong blocky structure; medium acid; gradual irregular boundary.
II C1 33 - 55 inches	Very pale brown (10YR 7/3 dry) to brown (10YR 5/3 moist) gravelly loam; massive structure; 20% gravel; slightly acid.
II C2 53 - 93 inches	Very pale brown (10YR 7/4 dry) to yellowish brown (10YR 5/8 moist) gravelly loam; massive structure; approximately 70% coarse gravel; neutral pH.

[1]Colors according to Munsell color system.

[2]Textures as determined by hydrometer method (Buoyoucos 1936).

Table 3. Soil profile description at ecounit 5.

O1 & O2 1 - 0 inches	Forest litter and partially decomposed litter.
Bir 0 - 6 inches	Light yellowish brown (10YR 6/4[1] dry) to dark yellowish brown (10YR 4/4 moist) friable loam;[2] granular structure; pH 5.7;[3] few sandstone coarse fragments; abrupt smooth boundary.
A2 & B2* 6 - 24 inches	Pale yellow (2.5& 7/4 dry) to light olive brown (2.5Y 5/4 moist) sandy loam; weak subangular blocky structure; pH 5.0; many sandstone coarse fragments; gradual irregular boundary.
B2t 24 - 30 inches	Pale yellow (2.5Y 7/4 dry) to light olive brown (2.5Y 5/4 moist) sandy clay loam; subangular blocky structure; pH 5.5; many sandstone coarse fragments; gradual irregular boundary.
C 30 plus inches	Pale yellow (2.5Y 7/4 dry) to light olive brown (2.5Y 5/4 moist) sandy clay loam; massive structure; pH 5.0; many sandstone coarse fragments.

*Horizon nomenclature in this profile may be questionable. This profile had generally poor development below the Bir.

[1]Colors according to the Munsell system.

[2]Textures as determined by the hydrometer method (Bouyoucos 1936).

[3]pH determined by saturated-paste method as described by Jackson (1958).

PRELIMINARY INVESTIGATIONS

The procedures used in this study are adapted from those used by Price (1965) and Boggie et al. (1958). Some preliminary investigations were necessary to adapt these procedures for use in the present study.

Preliminary chemical studies

The volume of soil into which an injected solution moved and the volume of solution that could be injected into the soil was determined. A 3/8-inch cold rolled steel rod was hammered into the soil, removed and a 3/8-inch aluminum tube inserted into the hole. A dowel was then inserted into the tube and forced one inch beyond the end of the tube. This cleaned soil out of the tube and also made a well at the bottom which increased the amount of solution in contact with the soil and aided percolation. Approximately 25 milliliters (ml.) of saturated potassium iodide solution followed by 25 ml. of water were poured down the tube. A vertical cut was then made through the soil profile at the point of injection. The face of the profile was sprayed with a solution of NaClO as household bleach, which oxidized I^- to I_2, and starch, to produce the characteristic iodine-starch blue color. The size of the blue colored surface indicated the spread of the KI solution in the soil. A sphere of approximately three inches in diameter was observed.

The soil's fine texture and accompanying low permeability caused much of the KI solution and water flush to remain in the aluminum tube even after very prolonged periods. The solution was pressured out of

the tube with a tire pump, but this forced the solution up the outer side of the tube to such an extent that depth placements of the isotope would have overlapped. Successively smaller aliquots of KI solutions and water flush were used until a volume which would infiltrate the soil in a few hours was reached; this value was five ml.

Selection of an isotope

Iodine-131 was chosen for use in this study for several reasons. (1) It is a beta and gamma ray emmitter. Both of these rays are easily detected, but alpha rays lack the capacity to penetrate thick plant tissues. In this study beta ray detection caused some difficulties which are discussed later. (2) Iodide ions are relatively mobile in the soil. They are fixed by non-living organic matter but are not held on the colloidal exchange sites (Raja and Babcock 1961). (3) The half-life of I-131 (8.05 days) is short enough to reduce radiation hazards, but is of sufficient length for short-term studies.

Plot selection

Ecounit 3 was chosen as a study site because of its proximity to the forest camp where a building to house the counting equipment was available. A relatively flat area with few trees and rather uniform soil was selected adjacent to ecounit 3. Ecounit 5 was selected as the site for adaptation of the technique in a stony soil because the profile is deep to consolidated bedrock.

Preliminary I-131 study

To test the technique in the field before applying it to the whole study, a plot was set up for injection prior to the main injection

date. The square-meter plot consisted of 25 aluminum tubes placed vertically six inches deep in the soil. The tubes were twelve inches long, 3/8-inch in diameter and of .049-inch wall thickness. A grid made from chicken wire was used to space the tubes evenly. The tubes were inserted by first hammering and withdrawing a 3/8-inch cold rolled steel rod and then the aluminum tube was placed in the hole. A 1/4-inch dowel was inserted into the tube and forced one inch beyond the end of the tube in order to make a well at the bottom of the tube to receive the solution and aid percolation into the soil.

The plot was then fenced with four-foot snowfence with a strand of barbed wire six inches above the snowfence. A padlock was placed on a gate and approved warning signs posted. The Atomic Energy Commission requires fencing and posting of areas in which radioactive materials are used.

Three millicuries of I-131 were diluted with 0.5N NaOH to 150 ml. in a plastic bottle. A piece of 3/16-inch lead sheet was placed around the bottle to partilly shield the radiation. On May 28, 5 ml. of 20 microcuries (uc) per ml. were injected into each of the aluminum tubes with an Adams Aupette, a syringe which dispenses a constant volume. A plastic cloth protected plants on the plot from contamination due to drops which may have accidentally escaped from the syringe needle. During both dilution in the laboratory and injection in the field all personnel wore laboratory coats, disposable plastic gloves, and pencil type ionization monitors. Left-over solution was stored in the laboratory for decay.

At one, two, three, and six days after injection plants in the plot were sampled for radioactivity. Leaves were removed from the plant,

cut into small pieces with scissors, and placed into a small plastic bag. The bag was rolled into a minimum volume and secured with a rubber band. A paper tag was stapled to the bag and the whole unit weighed. This sample was counted for 5 minutes under the window of a Geiger-Mueller (GM) tube (1.7-2.0 mg/cm^2 window thickness) connected to a scaler. Background count was a 5 minute count with no sample under the GM tube. It is recognized that the radioactive isotopes of uranium, potassium, and thorium as well as other radioactive isotopes occur naturally in the soil, but the amounts are small and probably do not add significantly to the background count (Libby 1955). Species sampled were Arnica (Arnica cordifolia), glacier lily (Erythronium grandiflorum), elk sedge (Carex geyeri), snowberry (Symphoricarpos albus), kinnikinnick (Arctostaphylos uva-ursi), creeping holly grape (Berberis repens) and western larch (Larix occidentalis). Average background count was about 65 counts per 5 minutes. The highest count for any of the species was 130 counts in 5 minutes, but most counts were much lower. Variation in background count overlapped most of the readings on the samples. Since the assumption was made that all of the species sampled rooted to at least six inches, it was apparent that the radioactive solution would have to be of greater activity; one millicurie (mc) per injection was assumed to be adequate for future work.

PROCEDURES

The procedures used in this study are a combination of those of Price (1965) and Boggie et al. (1958) along with the results of the preliminary studies as mentioned. The main study was conducted at ecounit 3, but a few plots were set up near ecounit 5 to adapt the technique to stony soils.

Tube placement and fencing

A series of meter-square plots were set up at ecounit 3 and a few at ecounit 5. At ecounit 3 tube depth placements of 6, 12, 24, 36, and 48 inches were replicated four times for a total of 20 plots. All plots were four meters apart. Later in the summer two half plots at 60 and 72 inches were put in at ecounit 3. An unreplicated series of plots at depths of 6, 12, 24, and 36 inches and just six tubes at 48 inches were set up at ecounit 5 to investigate the procedure in a stony soil. Procedures for placement of the tubes were identical to those used in the preliminary I-131 study. Prior to placement, six inches of one end of each tube was painted with a particular color, each color designating a depth. The painted six-inch portion of all tubes remained above the soil surface. For the half plots 1/4-inch steel pipe was used in place of aluminum tubing as it is cheaper.

At ecounit 5 placement of the tubes at depths greater than 36 inches was extremely difficult. When the iron rod was removed, the loose, coarse-textured soil filled in the holes. Consequently, only six tubes were placed at 48 inches.

A "come-along" hoist mounted on a tripod was used to remove

the iron rod from the 36 inch and deeper depths.

Both study areas were fenced and posted as in the preliminary study.

Isotope dilution and handling

On June 23, 500 mc of I-131 were received in two vials of 250 mc. each. The isotope was diluted with water to 250 ml. in each or two plastic bottles. This volume was sufficient for the 500 injections to be made that day at ecounit 3. Since the preliminary study showed 100 uc. per injection to be too low in activity, one mc. per injection was used. Injection of one ml. instead of five ml. reduced the hazard of such a large volume of radioactive solution, since a smaller container is easier to shield. The isotope was transported to Lubrecht Forest under a shield of lead sheets and lead bricks.

Field injection

As in the preliminary study, injections were made with the Adam's Aupette. Instead of a plastic cloth to protect plants from accidental drops, a masonite board with a handle and a hole in the center was held over each tube during injection (see Figure 1). After injection of one ml. of I-131 solution into each tube, four ml. of water were pipetted into the tube as a flush.

Sampling

A number of species were sampled and counted for several days after injection but no samples were radioactive above background radiation. Apparently the I^- had been oxidized to elemental iodine which is unavailable for plant uptake. One ml. of a 0.1M KOH, 0.5 M KI,

Figure 1. Injection. Note tube arrangement within plot and injection apparatus.

and 0.1 M $Na_2S_2O_3$ solution was added to each tube on one plot to convert the elemental iodine back to the reduced form. Within two days the plants in the treated plot became radioactive while plants on the other plots still gave only background counts. All plots were then given the above treatment. After two more days sampling was begun. The following species were sampled at ecounit 3:

Shrubs	Forbs
Snowberry (*Symphoricarpos albus*)	Arnica (*Arnica cordifolia*)
Huckleberry (*Vaccinium caespitosum*)	Strawberry (*Fragaria virginiana*)
Creeping holly grape (*Berberis repens*)	Yarrow (*Achillea millefolium*)
Kinnikinnick (*Arctostaphylos uva-ursi*)	Bedstraw (*Galium boreale*)
Woods rose (*Rosa woodsii*)	Pussy toes (*Antennaria rosea* and *A. parviflora*)

Grasses and sedges

Pinegrass (*Calamagrostis rubescens*)

Elk sedge (*Carex geyeri*)

A few other species were sampled, but the plants were not in sufficient number to obtain a measure of rooting depth (see Appendix IV). The species sampled at ecounit 5 are presented in Appendix V.

Five plants of each of the above species present in a plot were sampled; however, not all of these species occurred in all of the plots. In some of the deeper plots more than five samples were taken. Usually leaves were sampled instead of stems since a plant autoradiograph (Figure 2) showed concentration of the iodine in the leaves. As in the earlier study, the plant material was placed into a plastic bag and the bag and contents rolled into a small volume, and secured with a rubber

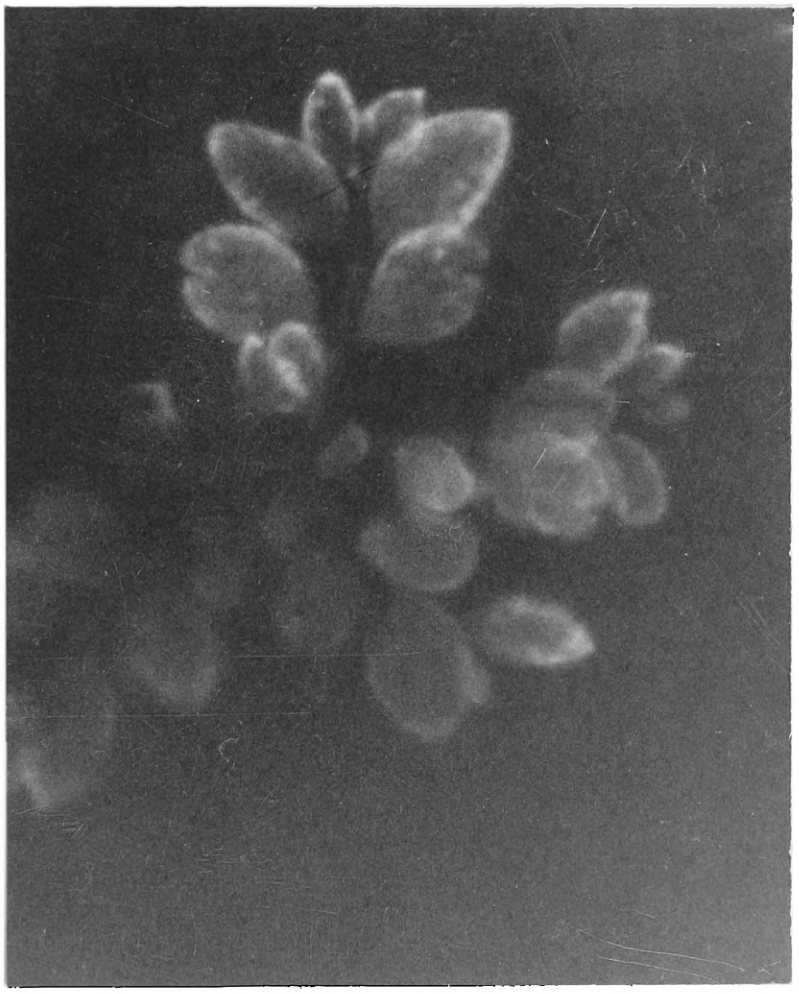


Figure 2. Plant autoradiograph. Note concentration of the isotope in the leaf margins (light portions).

band. A numerical symbol for the species, plot depth, and plant replicate was written on each bag.

Counting

Counting was accomplished with three scalers and three GM tubes. The sample was placed beneath the window of the GM tube and counted for five minutes. To determine the quantity of radioactivity in naturally occurring plants, samples of all species were taken outside of the fenced area and counted. The counts all agreed with those counts registered when no sample was under the GM tube (see Appendix VI). Background counts with no sample under the tube were taken several times on days when counting was being done.

RESULTS

Counting variation

Radiation counts recorded from plant samples ranged from background to several thousand counts per five minutes (c/5 min.) due to several factors. Because of the chemical difficulties of iodide oxidation discussed earlier, sampling was not begun until eleven days after injection. This time period corresponds to approximately 1.5 half-lives of I-131 and caused a considerable reduction in the number of counts obtained from plant samples. I-131 is both a beta and gamma ray emitter, but the counting efficiency of the Geiger tube for gamma rays is about 10 percent, while that for beta rays is approximately 90 percent. Therefore, most of the counts registered are beta rays. The thickness of the plastic bag, the thickness of the plant tissue, and the distance of the sample from the Geiger tube window appreciably affect the number of beta rays (which have low penetrating capacity) reaching the Geiger tube window. Thus, the number of counts registered for any one sample could vary greatly.

Another source of count variation is associated with the amount of soil contaminated with the isotope. The diameter of the sphere of contaminated soil at the bottom of the tube, as determined by autoradiography, was about ten centimeters. The autoradiograph was made by placing a sheet of Kodak ready pack no-screen X-ray film against the face of a vertical slice through the soil adjacent to an injection tube for a period of six hours (see Price 1965). Thus about 30 percent of the plot area at any depth was radioactively contaminated. It was there-

fore possible for plant roots to go between the spheres and not to pick up radioiodine. The amount of solution reaching the soil was probably fairly constant. Earlier investigation showed about 30 percent retention on the tube walls, but it was assumed that the four ml. water flush would remove any of the isotope clinging to the walls of the tubes.

Still another source of count variation was due to biological variation in rooting pattern among individual plants of a species because of age and genetic differences.

One other variation is due to lateral root extension which caused some plants to have their shoots in the plot and their roots outside of the plot. However, lateral extension was demonstrated to be no greater than one meter (see Appendix VII), so there was no chance of plants in one plot having their roots in a plot four meters distant.

Since such great variations in counting occurred, sample weighing was discontinued after the first day of counting.

As counting of each sample was initiated, the sample was turned in all directions under the Geiger tube until a position which registered maximum counts was reached.

The largest background count registered for five minutes was 119, and the average count was 80-100 c/5 min. The average count is a little higher than the background in the preliminary study because counting was done in a different building.

Only a few tissue samples of the approximately 1,000 counted were of questionable activity--seven samples had counts of 120 c/5 min. and 33 samples had counts between 120 and 130 c/5 min. Only two of the seven samples (both strawberry) were involved in a decision as to whether or

not the species rooted to a given depth; however, all of the questionable samples did contribute to the percent plants active. Choice of an arbitrary minimum count (120 c/5 min.) is not very accurate since many samples with counts lower than the chosen value may be radioactive. The chance of denoting an active sample as non-active was much greater than the converse since background counts as large as the maximum obtained (119 c/5 min.) were uncommon, so it is possible that several radioactive samples were called non-radioactive. Price (1965; personal communication) used statistical methods which involved resampling when necessary. Resampling of a particular plant was not possible in the present study as each plant was not identified throughout the sampling and counting procedures - only species, plot and plant replicate numbers were identified. In many cases (Carex for example) resampling would have been impossible since all of the above-ground tissue was removed at sampling time.

Rooting depths

If a sample from a species on any one plot was radioactive, it was assumed that the species is capable of rooting to the depth of isotope placement on that plot. The plot data for all species sampled at ecounits 3 and 5 appear in Appendices IV and V respectively. These data represent the fraction of the plants sampled found to be radioactive above background.

The percent of plants sampled active above background, for each species, may indicate the relative amount of rooting at the depth of isotope placement, since the more roots a plant has, the greater is the chance of the roots entering a sphere of contaminated soil and absorbing the isotope. Table 4 presents the rooting depth data for the more

abundant species as percent of plants sampled radioactive above background. Figure 3 shows the same data in graphic form.

Rooting on this site was surprisingly deep. Berberis repens, a low growing shrub, and Fragaria virginiana both rooted to 72 inches, a rather phenomenal depth. The roots of Achillea millefolium penetrated to only 12 inches. Arnica cordifolia and Calamagrostis rubescens rooted to 24 inches, while Symphoricarpos albus, Antennaria spp., and Galium boreale rooted to 60 inches. Intermediate in rooting depths were Vaccinium caespitosum which rooted to 36 inches and Rosa woodsii and Carex geyeri which rooted to 48 inches.

Some species (Douglas fir seedlings, for example) did not become radioactive in any of the plots, so it was assumed that these species rooted less than six inches deep (see Appendices IV and V).

Statistical analyses

An analysis of variance for Symphoricarpos albus was run to determine if there was a significant difference between treatments. This species was the only one in which twenty plants were sampled at each of the upper five depths. The data used in the test were percent plants active averaged for the replicate plots at each depth. Transformations were necessary to remove the effects of zeros and percentages (Steel and Torrie 1960), so the formula arc sine $\sqrt{1/2+x}$ was applied to each value (x = percent plants active averaged for four plots at each depth). A table of the analysis is shown in Table 5. No significant difference between treatment variance occurred at the five percent probability level. In order to obtain results of greater statistical significance, many of the causes of variation mentioned earlier must be subdued or eliminated.

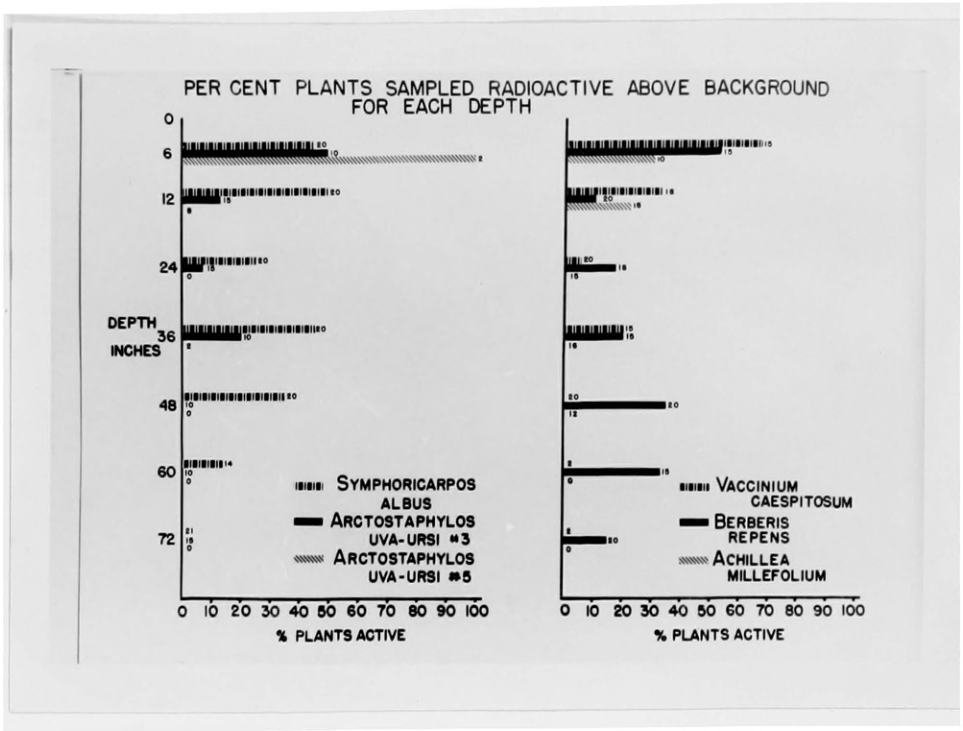
Table 4. The percent of the more abundant species at the two study areas (ecounits 3 and 5) that were radioactive above background, and the number of plants sampled

Species	Depth (inches) 6	12	24	36	48	60	72
Ecounit 3							
Arnica cordifolia	78(9)*	40(20)	27(15)	0(15)	0(15)	--	--
Carex geyeri	94(16)	45(20)	21(16)	28(18)	35(20)	0(8)	0(11)
Symphoricarpos albus	45(20)	50(20)	25(20)	45(20)	35(20)	14(14)	0(21)
Arctostaphylos uva-ursi	50(10)	13(15)	7(15)	20(10)	0(15)	0(2)	0(5)
Berberis repens	53(15)	10(2)	17(18)	20(15)	35(20)	33(15)	15(20)
Fragaria virginiana	40(15)	80(5)	50(10)	13(11)	7(15)	0(3)	18(6)
Calamagrostis rubescens	80(10)	62(16)	60(10)	0(10)	0(2)	--	--
Achillea millefolium	30(10)	22(18)	0(15)	0(16)	0(12)	--	--
Galium boreale	79(10)	42(19)	25(20)	10(20)	7(14)	13(15)	0(23)
Vaccinium caespitosum	67(15)	33(18)	10(20)	20(15)	0(20)	0(2)	0(2)
Rosa spp.	57(7)	14(7)	7(14)	21(14)	25(8)	--	0(3)
Antennaria spp.	82(11)	20(5)	--	33(6)	14(7)	67(3)	--

Table 4 (continued)

Species	Depth (inches) 6	12	24	36	48	60	72
Ecounit 5							
Arnica cordifolia	100(10)	20(5)	50(10)	0(1)	0(1)	----	----
Carex spp.	----	0(10)	----	0(1)	----	----	----
Xerophyllum tenax	100(5)	80(5)	40(5)	0(5)	0(2)	----	----
Arctostaphylos uva-ursi	100(2)	0(8)	----	0(2)	----	----	----
Calamagrostis rubescens	60(5)	90(10)	0(5)	0(3)	0(1)	----	----
Vaccinium membranaceum	50(10)	0(10)	0(7)	0(10)	0(6)	----	----

*The first number represents the percent of the plant samples that were radioactive above background and the second (in parenthesis) the number of plants sampled. A blank indicates that no plants were sampled.



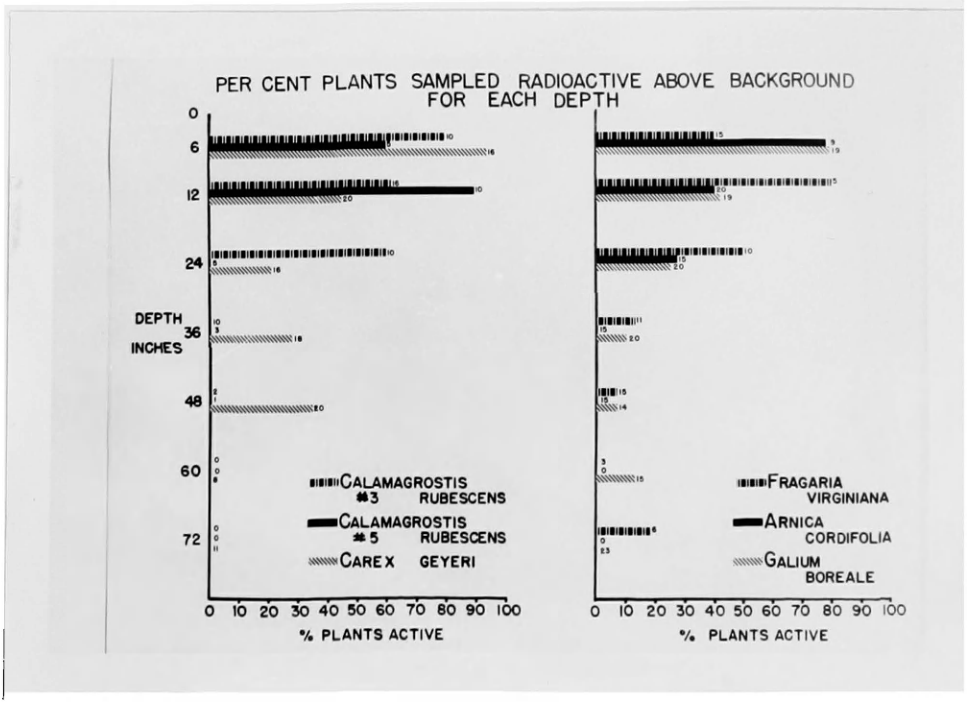


Figure 3. Percent plants sampled radioactive above background for each depth. Number at end of each bar represents the number of plants sampled.

The difference in percent plants active for each species at the various depths was tested for significance by Chi-Square (see Table 6). A significant Chi-Square was found for all species except Calamagrostis rubescens and Achillea millefolium. Both of these species are relatively shallow rooted and the differences in amount of rooting at each of the depths was quite small (see Figure 3).

Table 5. Analysis of variance for Symphoricarpos albus - first five depths

Category	df	Sum of Squares	Variance	Sample F	F.05
Replicate	3	0.20	0.07	0.41	3.49
Depth	4	0.32	0.08	0.47	3.26
Error	12	2.08	0.17	----	----
Total	19	2.60	----	----	----

Table 6. Chi-Square tests between depths, within species probability level 5%. (Depths with 0% plants active omitted).

Species	[1]Sample X^2	[2]Theoretical X^2	df
Arnica cordifolia	29.3	6.0	2
Carex geyeri	75.0	9.5	4
Symphoricarpos albus	10.0	9.5	4
Arctostaphylos uva-ursi	49.8	7.8	3
Berberis repens	44.0	9.5	4
Fragaria virginiana	92.0	9.5	4
Calamagrostis rubescens	3.6	6.0	2
Achillea millefolium	1.2	3.8	1
Galium boreale	105.0	9.5	4
Antennaria spp.	77.4	7.8	3
Vaccinium caespitosum	67.8	7.8	3
Rosa woodsii	47.7	9.5	4

[1]Sample X^2 derived as follows: observed = percent plants active for each depth; expected = average of percent plants active for each depth.

[2]Theoretical X^2 is from a table of Chi-Square.

DISCUSSION

Rooting depths

Rooting depths at ecounit 3 were somewhat surprising. Galium boreale, for example, is a small, succulent, perennial plant, but it rooted to 60 inches. Berberis repens, a low growing shrub with few, leathery leaves rooted to 72 inches as did strawberry. It appears as if these plants are rooting deeply to obtain soil moisture in the dry summer months, however some investigators (Loomis and Ewan 1936) believe hydrotropism does not occur appreciably under field conditions. It appears that hydrotropism, at least to a certain extent, may affect rooting depths on this site, although deep rooting is probably largely controlled by genetic factors.

Comparisons to other rooting depth studies

The percent plants active is a measure of the amount of rooting at a depth since the frequency of roots penetrating the radioactively contaminated spheres increases with larger numbers of roots.

A progressively decreasing density of roots from the surface of the soil profile, downward would normally be expected. Several of the species sampled did not follow this pattern, but had less rooting (lower percent plants active) in the 12-36 inch depths and an increase in rooting at 48 inches (see Figure 3). Rooting of these species decreased progressively from 48 inches to 72 inches. The 12-36 inch depths are analogous with the thickness and depth of the B2t horizon (see Appendix III). Apparently, the reduction in rooting is due to the fine texture and blocky to prismatic structure in this horizon,

causing reduced root development. The roots tend to channel along the ped surfaces and lateral root extension appears to be minimal in this horizon (observed in soil pits). Duncan (1941) and Diebold (1933) noticed the same phenomenon in fine-textured subsoils (see literature review). Cox (1959) found the subsoil of the Lubrecht series to be poorest medium for rooting of three soils studied on Lubrecht Forest.

Several of the species sampled were also studied by other investigators. Berndt and Gibbons (1958) studied root distribution of several tree and understory species in Colorado pine types by mechanical excavation (see literature review). They found the maximum depth of Arctostaphylos uva-ursi to be three feet on a gravelly sandy loam; this species also rooted to three feet in the fine-textured soil of the present study. Apparently the compact B horizon of the Lubrecht soil did not appreciably affect rooting depths of this species.

Weaver (1919) studied several species of a forest community in the Colorado Rocky Mountains. He found the maximum rooting depths for Arctostaphylos uva-ursi, Symphoricarpos, and Fragaria virginiana to be 46 inches, 65 inches, and 14 inches respectively. In the present study these species rooted to 36 inches, 60 inches and 72 inches respectively. The site in Weaver's study is not described, but these results show how a species can vary in rooting depth from one site to another. The snowberry (Symphoricarpos) in Weaver's study was a different species than that in the present study.

The study by Boggie et al. (1958) included Achillea millefolium, four species of Carex, and two species of Galium. Achillea rooted to approximately 24 inches in sand as compared to 12 inches in the present

study. The sand is probably less restrictive to rooting, and perhaps drier, than the fine-textured soil of the Lubrecht series. Carex arenaria rooted to 40 inches in sand, but the other Carex species rooted to only four inches in a stony loam, overlying an indurated layer. Carex geyeri rooted to 48 inches in the present study. This demonstrates that Carex has a wide variation in rooting depths, depending largely upon edaphic factors. Galium hercynicum rooted to 24 inches on one site and 12 inches on another site, while Galium vernum rooted to 24 inches. In the present study Galium boreale rooted to 60 inches. Galium also appears to vary widely in rooting depth from one site to another.

APPLICATION

Rooting depths and soil moisture

Since evaporation of moisture from forested soils with a litter horizon is nearly negligible, transpiration accounts for almost all of the water loss from these soils, with the exception of deep percolation. A correlation between root distribution and soil moisture loss may exist for the study site.

According to Kramer (1949, p. 121) extension of roots through the soil is essential for absorption of water. Hydrotropism at this relatively zeric site may affect rooting; however, Loomis and Ewan (1936) demonstrated that most plants do not show hydrotropism. The environment probably selects genetically for deeply rooting species, and deep rooting on this site may partially be related to genetics. In spite of the findings of Loomis and Ewan, hydrotropism may affect rooting depths at this site.

Several studies have been conducted which relate soil moisture and rooting depth. Doss et al. (1960) and Bennet and Doss (1960) studied the effects of soil moisture levels on root distribution of several forage species by three methods. One method involved measuring root distribution indirectly by soil moisture depletion. The two other methods involved direct observation of the roots. The results of the study indicated shallower rooting on sites high in soil moisture than on sites low in soil moisture.

Rooting depth and soil moisture removal have also been compared by Wood (1960). Soil moisture removal followed the pattern of root dis-

tribution. Positions in the profile from which large quantities of moisture were removed had many more roots than points of low moisture removal.

In some grassland communities, deeply rooting species such as sagebrush (Artemisia spp.) utilize the deeper soil moisture while the grasses and forbs use moisture close to the soil surface. This is illustrated by the following data obtained at the end of the growing season on a fairly uniform site (Rowe and Reiman 1961). Brush and grass cover are compared to moisture depletion using field moisture capacity (FMC) and permanent wilting percentage (PWP) as reference points.

Table 7. Soil moisture depletion by brush and grass, using field moisture capacity (FMC) and permanent wilting percentage (PWP) as reference points.*

	Moisture less than:	Depth in profile: (inches)
Brush	FMC	144
	PWP	84
Grass	FMC	84
	PWP	26

*From Rowe and Reiman 1961.

Shrub species removed moisture to much greater depths than did grass species.

These studies indicate that the position in the profile of moisture absorbing roots corresponds with zones of soil moisture depletion.

Soil moisture data collected at ecounit 3 (T. Nimlos, Univ. of Montana, personal communication) indicate an earlier moisture depletion

at the 3, 10 and 42-inch depths than at 19 and 30 inches. The latter two depths are in the zone of limited rooting (see Figure 3) and a correlation between the amount of rooting (percent plants active) and soil moisture depletion might be assumed for this site.

Depths for measuring soil moisture

As previously mentioned, soil moisture is being measured with Colman fiberglass resistance units at each of the ecounits (T. Nimlos, Univ. of Montana, personal communication). Since root distribution and soil moisture depletion appear to be well correlated, the distribution of rooting on the study site should indicate at what depths soil moisture should be measured.

The percent of plants sampled for each species found to be radioactive above background is probably an indication of the relative number of roots of a species at the depth of isotope placement. The more roots a plant has, the higher is the frequency of its roots coming into contact with the spheres of contaminated soil and picking up the isotope.

Total rooting intensity at each depth may be determined from the percent plants active above background for all species. Table 8 shows the fraction of plants of the 12 major species sampled found to be active above background for each depth, the percent plants active, and the percent of the total rooting in the upper 72 inches of the profile to each depth. A curve of the percent plants active by depths appears in Figure 4. The high percent plants active at 60 inches is mainly due to one species. Of the 11 out of 62 plants sampled found to be active above background, five were Berberis repens. B. repens is not a dominant species on the site, however. This example points out one error

Table 8. Fraction of plants of the twelve main species sampled found to be active above background, percent plants active, and percent of total rooting in the upper 72 inches of the profile to each depth.

Depth	Fraction of plants active	Percent plants active[1]	Percent of total rooting[2]
6	99/157	63	36
12	66/183	36	57
24	36/182	20	68
36	31/170	18	78
48	26/159	16	88
60	11/62	18	97
72	4/91	4	100

[1]This is a measure of rooting intensity.

[2]These figures are the result of dividing the cumulative percent plants active from the surface to each depth by the total percent plants active from the surface to 72 inches.

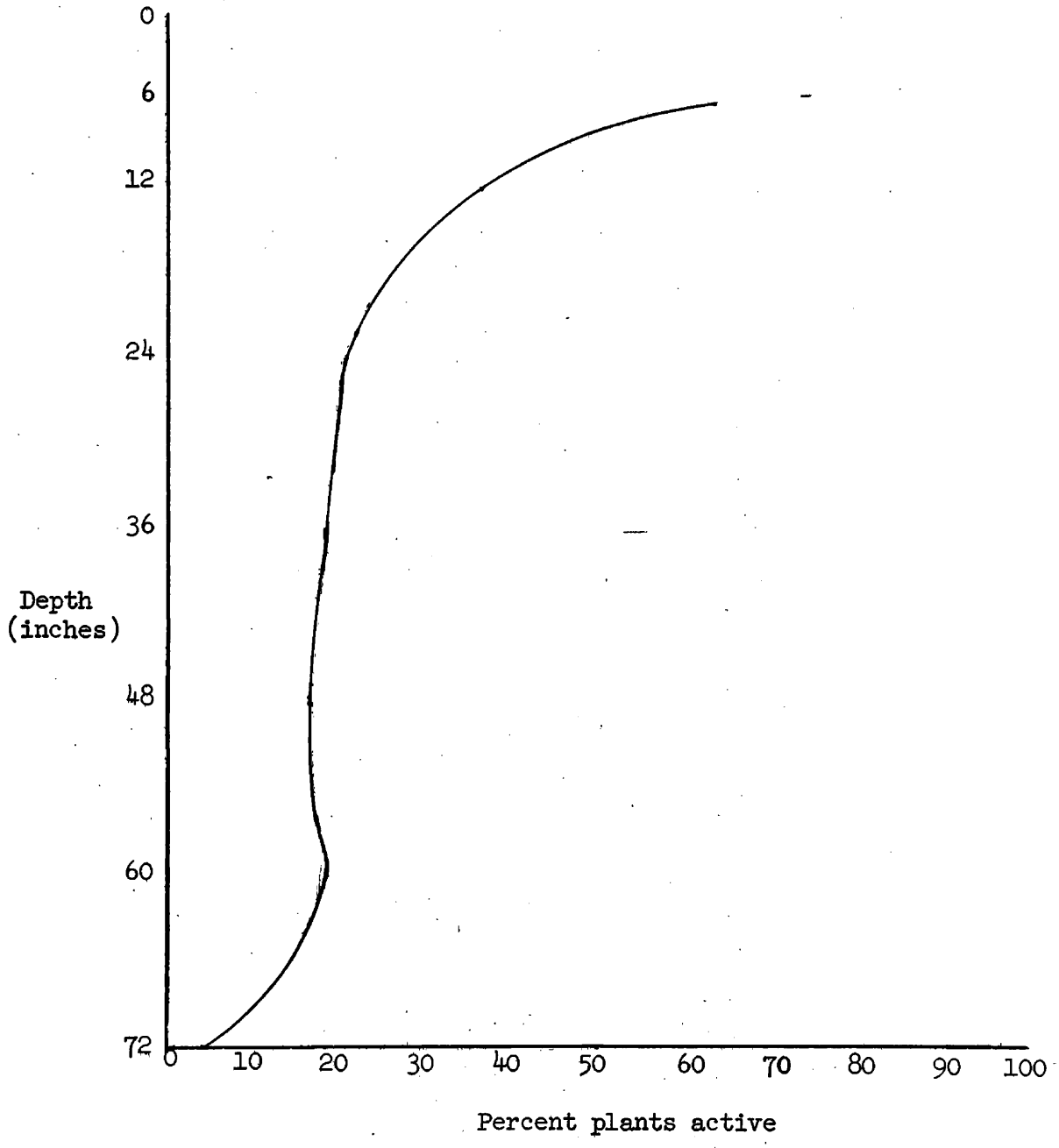


Figure 4. Total percent plants of main species active above background for each depth.

in the curve. One root is not necessarily comparable to another root because all species are not of the same density and dominance on the site, nor are all species equal in moisture utilization. Despite this error, the curve indicates at what depths rooting is concentrated.

Figure 5 shows a curve of the percent of total rooting in the upper 72 inches of the profile to each depth. The ecological characterization of a site may require that a certain percentage of the rooting be included in the zone in which soil moisture is measured. The maximum depth necessary to include a given percentage of the rooting can be read from Figure 5.

Cox (1959) also conducted a root distribution study on the Lubrecht soil using a trench method (described in the literature review). He found that about 87 percent of the roots were concentrated in the upper two feet of the soil profile. The curve in Figure 5 shows about 68 percent of the roots in the surface two feet as calculated by this method.

The difference in the results may be due to one of several reasons: (1) the method of Cox may not be conducive to locating many of the fine roots; (2) the curve in Figure 5 may not be accurate because of insufficient sampling; (3) tree roots that extended into the study area were not measured in the present study, but were included in the results of Cox.

Limitations

Count rate from radioactive samples should not be considered a meaningful measurement for several reasons. The assumption may be made that I-131 uptake accompanies moisture uptake because the same root

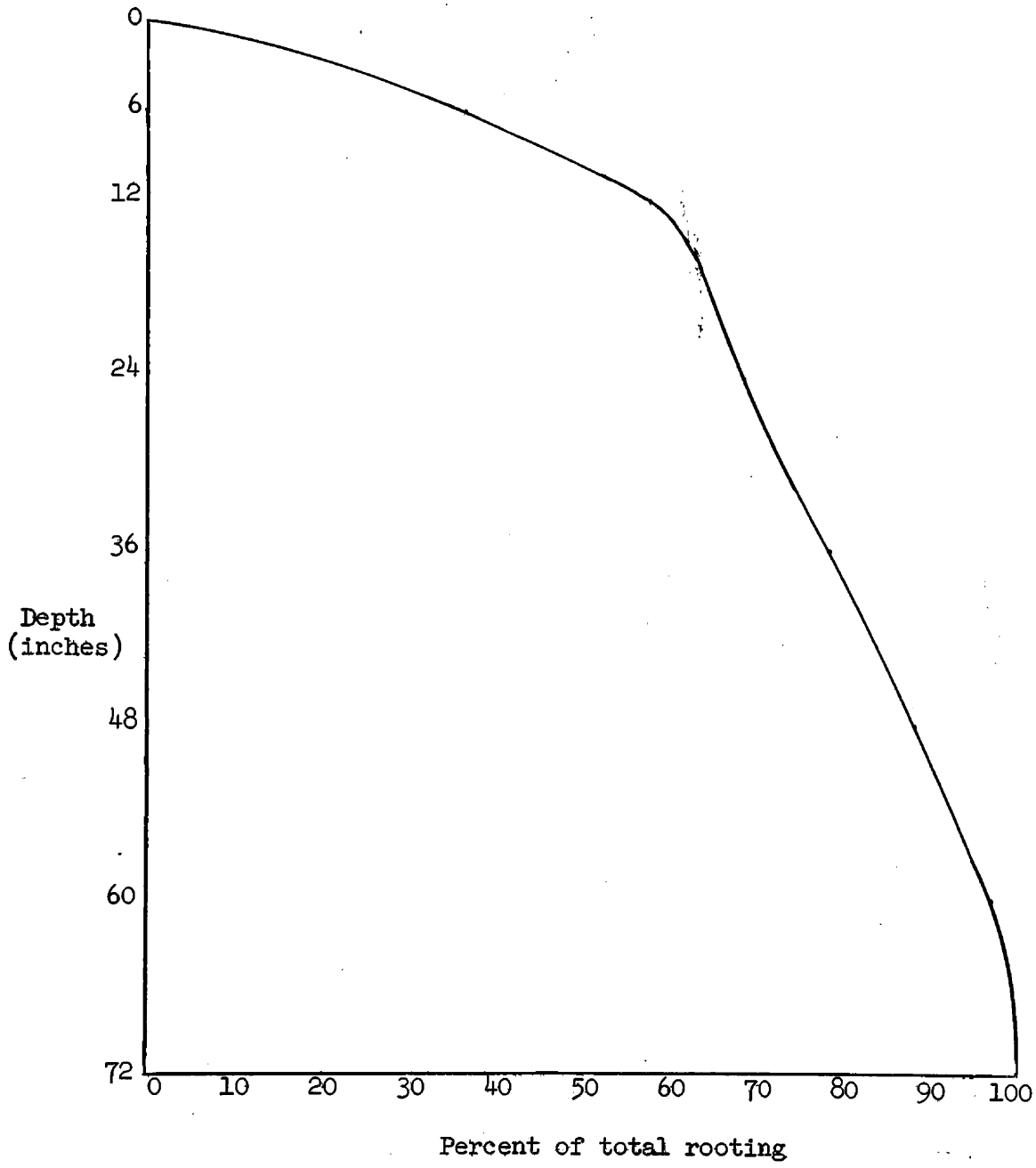


Figure 5. Percent of total rooting in the upper 72 inches of the profile to each depth.

zones are involved in each process. The rooting intensity profile in Figure 5 may give an indication as to where in the soil profile moisture is being absorbed, but it should not be interpreted quantitatively. Ion uptake and water absorption do not have a linear relationship (Brouwer 1965). Also, iodine uptake in particular is a heritable character (Butler and Glenday 1962) and varies between species and between strains (Johnson and Butler 1957). Therefore, even if quantitative counts of radioactivity in tissue can be made, count rate would not necessarily correlate with water absorption.

AUXILIARY STUDIES

To determine how soon after injection sampling should begin, a rose plant was encircled with injection tubes so that all portions of the plant could become radioactive. Samples were taken after injection for nine consecutive days and also on the twelfth day. The results of these measurements are shown graphically in Figure 6. Because of counting variation a great deal of scatter occurs in the points, but it appears as if plants should be sampled about six days after injection. Price (1965) found that plants should be sampled five days after injection. A similar procedure should be tried on several species, however, because not all plants have the same capacity to absorb radioiodine (Johnson and Butler 1957).

A study of lateral root extension resulted in very sketchy information because of insufficient sampling (see Appendix VII). Injection tubes were placed in a row about one meter long. A set of such tubes was placed at 6, 12, 24, 36, and 48-inch depths. After injection of the isotope the plants were sampled at various distances from each row. If a plant was radioactive at any one distance from the line of isotope placement it was assumed that its roots extended laterally to that distance. Figure 7 shows maximum lateral root extension by species for all depths.

The hydraulic method of studying roots (as described in the literature review) was applied to the pits opened for soil descriptions to further substantiate root phenomena interpreted from the isotope study. Visual observation of the intermingled root systems of all species again displayed the difficulty of studying roots by mechanical

methods. The most important observation was the extent of lateral rooting, especially above the B2t horizon. The roots may extend laterally to find the path of least resistance through the B2t. Extensive horizontal rooting of some species made study of any one plant very difficult by the hydraulic method. Observations of large root numbers in the surface portion of the profile were in agreement with the results of Cox (1959).

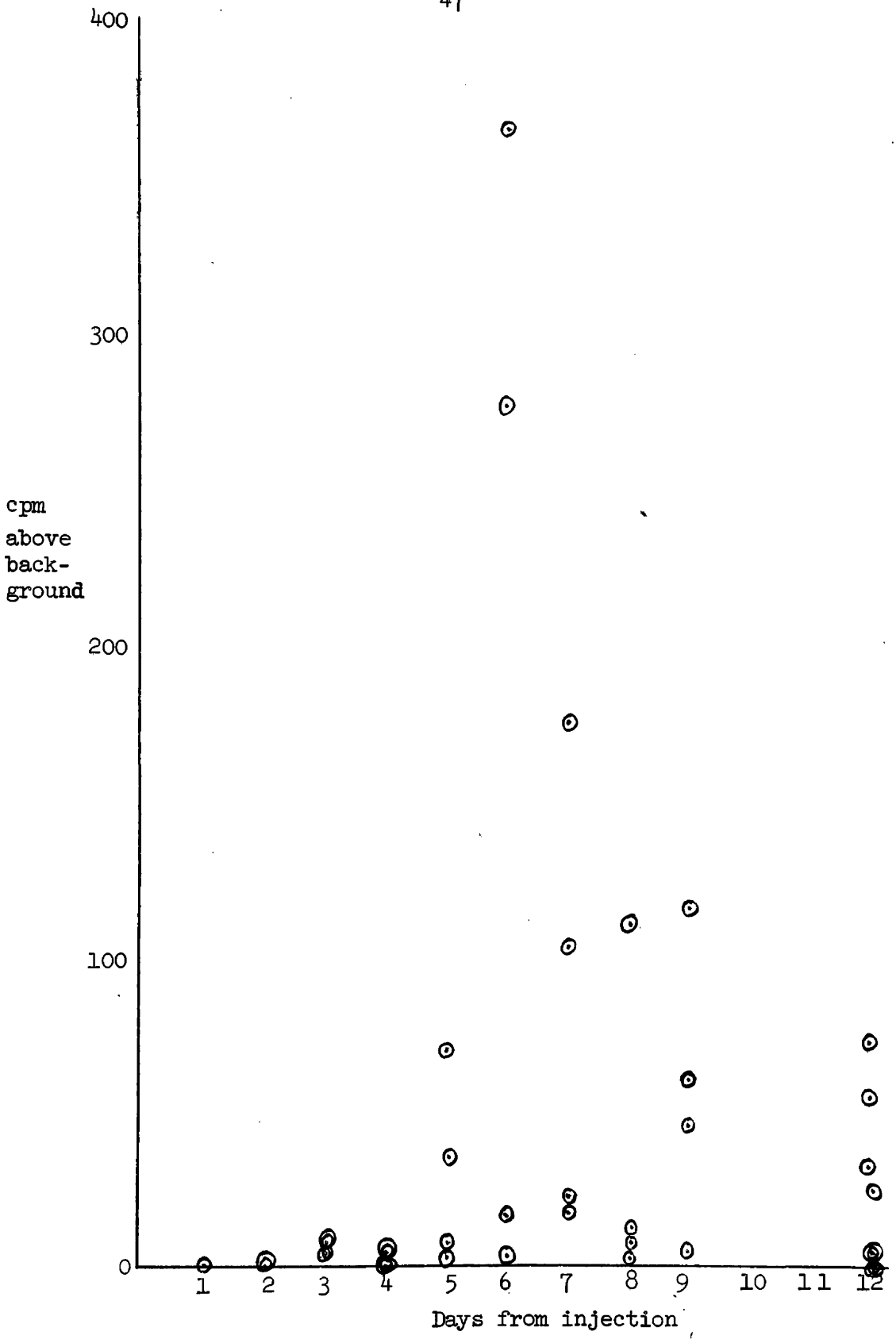


Figure 6. Activity-time curve of an injected rose plant--counts per minute (cpm) above background.

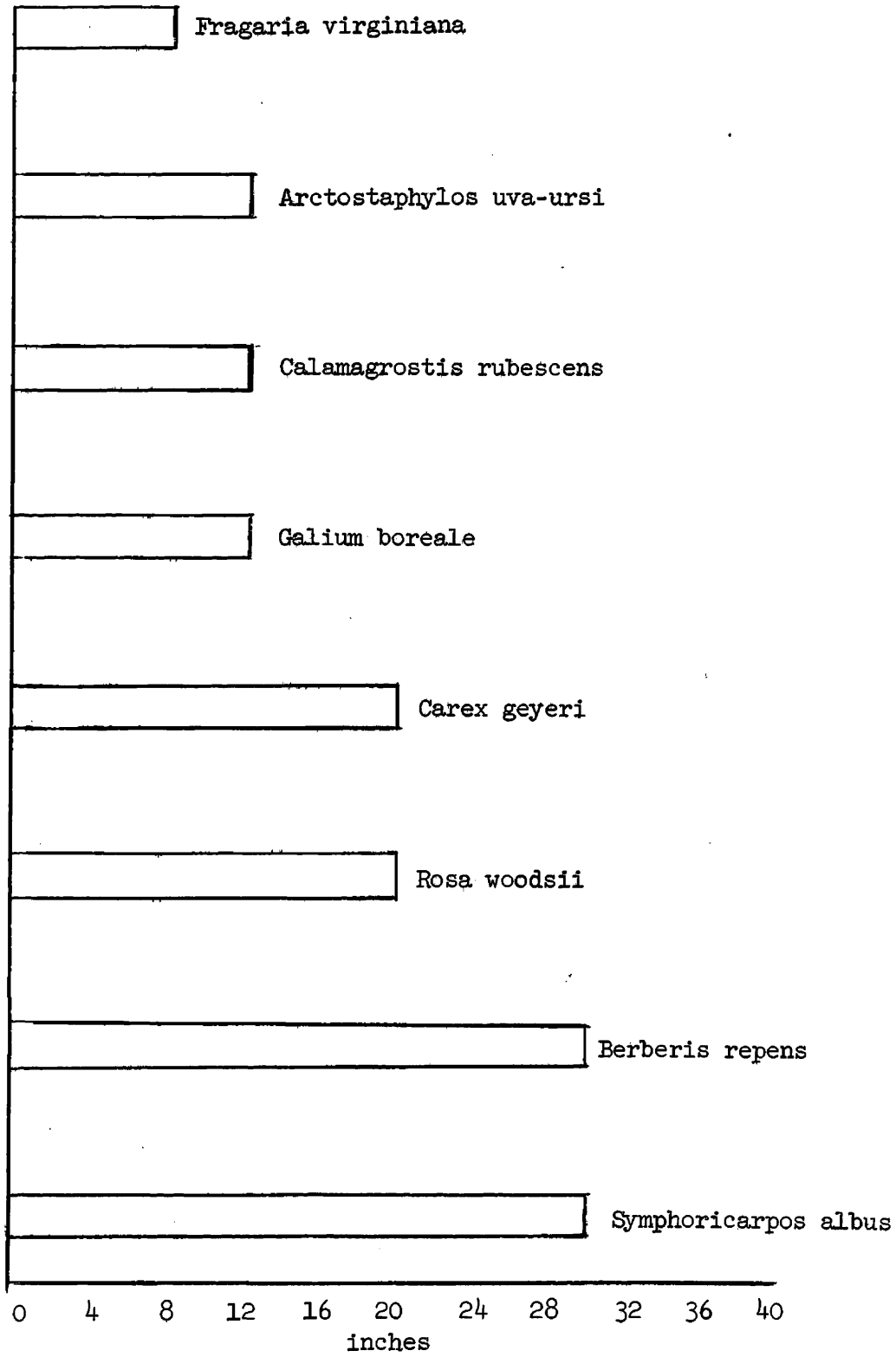


Figure 7. Lateral root extension

SUGGESTIONS FOR IMPROVEMENT OF FUTURE ROOTING STUDIES

As already mentioned, there were many sources of variation which prohibited quantitative interpretation of radioactivity in the tissue samples. A gamma scintillation detector with a well-type scintillation crystal is a more desirable counting instrument since it reduces background count and is more efficient in counting gamma rays than the Geiger tube.

To reduce the number of roots passing between contaminated spheres in the soil, about 36 tubes should be placed in each plot. The use of too many tubes, however, may cause excessive mechanical root injury.

More radioactive solution should be used to enlarge the contaminated sphere. A deeper well at the bottom of the injection tube may be made to accommodate the extra solution.

Sampling enough plants of each species is a problem on sparsely populated sites, or when plant distribution is not random. In either of these cases, larger and perhaps rectangular shaped plots, which include more species when the vegetation is not randomly distributed, are necessary.

The lateral root extension study will be improved by doubling the length of the row of injection tubes from one to two meters so that more plants of each species can be sampled.

SUMMARY AND CONCLUSIONS

Rooting depths of several understory species were determined by injection of radioiodine into the soil at various depths and measuring uptake in the leaves. The objectives of the study were (1) to adapt a technique of root study for use in fine-textured soils and stony soils, and (2) to determine the maximum depth to which soil moisture measurements should be made.

The procedures used in this study were adapted from those of Price (1965) and Boggie et al. (1958), and also included modifications developed during investigations conducted prior to the main study.

Tube placement was relatively easy in the fine-textured soil, but it was very difficult to place tubes deeper than 48 inches in the stony soil. Reduced volumes of injected solution were necessary in the fine-textured soil because of slow percolation.

Statistical analysis of the results showed large within treatment variation. A lack of precision in counting methods, considerable distance between radioactive spheres in the soil through which roots could pass uncontaminated, and normal biological variations together produced highly variable results.

Genetic selection for deeply rooting plants is most likely to be the cause of the deep rooting found in the fine-textured soil. Several of the species rooted to surprisingly great depths. Galium boreale, a small, succulent, perennial plant rooted to 60 inches. Berberis repens, a low growing shrub with few, leathery leaves rooted to at least 72 inches.

A curve for determining the maximum depth for making soil moisture measurements in the fine-textured soil was derived. Reading from this curve (Figure 5): approximately 88 percent of the roots would be included if soil moisture is measured to 48 inches. Measuring to 60 inches would increase the amount of roots included to about 97 percent.

Several auxiliary studies were also conducted. An autoradiograph of a plant showed concentration of the isotope in the leaf margins, thereby validating the sampling of leaf portions of plants. An investigation of lateral rooting shows maximum lateral root extension to be about 30 inches. To determine the best time for sampling following injection, a single rose plant was surrounded by isotope placements. Sampling on several consecutive days showed maximum isotope uptake occurring five to six days following injection.

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APPENDICES

Appendix I. Vegetation analysis for the site at ecounit 3 (24 quadrats).

	Quadrat of occurrence	Frequency %	Number plants	Relative frequency %	Relative density %
Carex geyeri	17	71	49	9	7
Carex spp.	20	83	154	10.5	22
Symphoricarpos albus	22	92	89	11.5	12.5
Arctostaphylos uva-ursi	15	63	51	8	7
Berberis repens	19	79	62	10	9
Fragaria virginiana	15	63	34	8	5
Calamagrostis rubescens	9	37	17	5	2
Stipa richardsoni	2	8	11	1	1.5
Achillea millefolium	14	58	50	7.5	7
Galium boreale	18	75	40	9.5	6
Antennaria spp.	7	29	50	3.5	7
*Pseudotsuga menziesii	2	8	2	1	0
Vaccinium caespitosum	17	71	62	9	9
Rosa woodsii	6	25	8	3	1
Geranium viscosissimum	1	04	1	0.5	0
Aster spp.	5	20	25	2.5	4
Linneae borealis	1	4	3	0.5	0
	190		708	100.0	100.0

*Seedlings less than six inches high

Appendix II. Vegetation analysis for the site at ecounit 5 (six quadrats)

	Quadrats of occurrence	Frequency %	Number plants	Relative frequency %	Relative density %
Arnica cordifolia	3	50	11	13	16
Chimaphila umbellata	5	84	15	22	21
Xerophyllum tenax	2	33	7	9	10
Arctostaphylos uva-ursi	1	17	1	4	1
Linnaea borealis	2	33	2	9	3
Calamagrostis rubescens	2	33	5	9	7
*Pseudotsuga menziesii	4	67	13	17	18
Vaccinium membranaceum	4	67	17	17	24
	23		71	100	100

*Seedlings less than six inches high.

Appendix III. Soil profile descriptions for ecounit 3.

Pit No. 1

O1 & O2 2-0 inches	Forest litter and partially decomposed litter.
A2 0-5 inches	Pale brown (10YR 6/3[1] dry) to dark brown (10YR 4/3 moist) silt loam;[2] weak, medium subangular blocky structure; pH 6.1;[3] clear, smooth boundary.
A2 & B2 5-10 inches	Light yellowish brown (10YR 6/4 dry) to dark yellowish brown (10YR 4/4 moist) clay loam; moderate, medium-fine subangular blocky structure; less than 1% gravel; pH 5.7; clear smooth boundary.
B2t 10-18 inches	Light yellowish brown (10YR 6/4 dry) to dark yellowish brown (10YR 4/4 moist) silty clay; strong, medium-coarse subangular blocky structure; pH 5.5; abrupt irregular boundary.
B3 18-36 inches	Very pale brown (10YR 7/4 dry) to yellowish brown (10YR 5/6 moist) clay loam; weak to moderate, medium subangular blocky structure breaking down to fine blocky structure; numerous clay pockets and clay skins on peds; many concretions; pH 5.6; gradual, irregular boundary.
IIC1 36-60 inches	Reddish yellow (7.5YR 6/6 dry) to strong brown (7.5YR 5/6 moist) loam; massive structure; 30-40% stratified gravel; pH 6.1; gradual irregular boundary.
IIC2 60-90 inches	Reddish yellow (7.5 YR 6/6 dry) to strong brown (7.5YR 5/6 moist) sandy clay loam; massive structure; 70-80% cobble and gravel; pH 6.8.

[1]Colors according to the Munsell system.

[2]Textures as determined by the hydrometer method (Bouyoucos 1936).

[3]pH determined by saturated-paste method as described by Jackson (1958).

Appendix III (continued)

Pit No. 2

O1 & O2 3-0 inches	Forest litter and partially decomposed litter.
A2 0-5 inches	Pale brown (10YR 6/3 dry) to dark yellowish brown (10YR 3/4 moist) silt loam; weak, medium subangular blocky structure breaking down to fine blocky structure; pH 5.7; clear, smooth boundary.
A2 & B2 5-12 inches	Light yellowish brown (10YR 6/4 dry) to dark yellowish brown (10YR 4/4 moist) clay loam; moderate, medium subangular blocky structure; less than 5% coarse fragments; pH 5.7; clear, irregular boundary.
B21 12-18 inches	Light yellowish brown (10YR 6/4 dry) to brown (10YR 5/3 moist) clay loam; moderate, medium blocky structure; thick clay films; pH 5.8; gradual, irregular boundary.
B22t 28-46 inches	Light yellowish brown (10YR 6/4 dry) to dark yellowish brown (10YR 4/4 moist) silty clay; moderate, medium blocky structure; less than 5% gravel; pH 6.3; clear, irregular boundary.
IIC1 46-62 inches	Very pale brown (10YR 7/4 dry) to yellowish brown (10YR 5/8 moist) loam; massive structure; 15% stratified gravel; pH 6.2.
IIC2 62-90 inches	Very pale brown (10YR 7/4 dry) to yellowish brown (10YR 5/8 moist) loam; massive structure; 50-60% gravel; pH 6.9.

Appendix III (continued)

Pit No. 3

O1 & O2 2-0 inches	Forest litter and partially decomposed litter.
A2 0-5 inches	Pale brown (10YR 6/3 dry) to dark brown (10YR 3/3 moist) silt loam; weak, medium subangular blocky structure; pH 6.4; clear, smooth boundary.
A2 & B2 5-12 inches	Light yellowish brown (10YR 6/4 dry) to dark yellowish brown (10YR 4/4 moist) silty clay; moderate medium subangular blocky structure; pH 5.6; abrupt, irregular boundary.
B2t 12-33 inches	Light yellowish brown (10YR 6/4 dry) to dark yellowish brown (10YR 4/4 moist) clay; medium to strong, coarse prismatic structure breaking down to strong, medium blocky structure; thin clay films; pH 6.3; gradual irregular boundary.
IIC1 33-53 inches	Very pale brown (10YR 7/3 dry) to brown (10YR 5/3 moist) loam; massive structure; 20% stratified gravel; pH 6.6.
IIC2 53-93 inches	Very pale brown (10YR 7/4 dry) to yellowish brown (10YR 5/8 moist) loam; massive structure; about 70% gravel; free lime present; pH 7.4.

Appendix III (continued)

Pit No. 4

O1 & O2	Forest litter and partially decomposed litter.
A2 0-5 inches	Very pale brown (10YR 7/3 dry) to dark brown (10YR 4/3 moist) silt loam; weak, medium subangular blocky structure; pH 6.3; clear, smooth boundary.
A2 & B2 5-11 inches	Very pale brown (10YR 7/3 dry) to dark brown (10YR 4/3 moist) clay loam; moderate, medium and fine subangular blocky structure; pH 5.7; clear, smooth boundary.
B21t 11-31 inches	Very pale brown (10YR 7/4 dry) to yellowish brown (10YR 5/4 moist) silty clay; strong, coarse subangular blocky structure breaking down to medium subangular blocky structure; coatings of light-colored material on peds and stains of clay and organic matter on peds; pH 5.6; clear, irregular boundary.
B22t 31-41 inches	Very pale brown (10YR 7/4 dry) to light yellowish brown (10YR 6/4 moist) clay; strong, medium subangular blocky structure; pH 6.3; clear, irregular boundary.
IIC1 41-68 inches	Very pale brown (10YR 8/3 dry) to light yellowish brown (10YR 6/4 moist) loam; massive structure; gravel present; pH 6.0.
IIC1 68-90+ inches	Very pale brown (10YR 8/3 dry) to pale brown (10YR 6/3 moist) loam; massive structure; about 50% stratified coarse fragments with lime on undersides of fragments; pH 6.2.

Appendix IV. Fraction of plants sampled found to be active above background at ecounit 3.

Species	Depths (inches) 6	12	24	36	48	60	72
Arnica cordifolia							
**1	5/5	1/5	2/5	0/5	*---	---	---
2	3/3	1/5	2/5	0/5	---	---	---
3	1/1	2/5	0/5	---	0/5	---	---
4	---	4/5	---	0/5	0/1	---	---
Carex geyeri							
1	4/5	1/5	0/5	1/5	0/5	0/5	0/6
2	5/5	3/5	0/5	2/5	3/5	0/3	0/5
3	1/1	4/5	1/4	2/5	4/5	---	---
4	5/5	1/5	3/5	0/3	0/5	---	---
Symphoricarpos albus							
1	1/5	5/5	1/5	2/5	0/5	1/5	0/12
2	3/5	1/5	1/5	3/5	0/5	1/9	0/9
3	3/5	1/5	2/5	3/5	3/5	---	---
4	2/5	3/5	1/5	1/5	4/5	---	---
Arctostaphylos uva-ursi							
1	---	1/5	0/5	---	0/5	0/2	0/5
2	---	1/5	---	0/5	0/5	---	---
3	2/5	0/5	0/5	2/5	---	---	---
4	3/5	---	1/5	---	0/5	---	---
Berberis repens							
1	---	2/5	0/5	0/5	1/5	2/6	3/13
2	2/5	1/5	2/5	0/5	3/5	3/9	0/7
3	4/5	0/5	1/5	3/5	0/5	---	---
4	2/5	0/5	0/3	---	3/5	---	---

Appendix IV (continued)

Species	Depths (inches) 6	12	24	36	48	60	72
Fragaria virginiana							
**1	0/5	4/5	*---	---	---	0/3	0/3
2	---	---	2/5	0/5	0/5	---	1/3
3	2/5	---	---	1/3	0/5	---	---
4	4/5	---	3/5	1/3	1/5	---	---
Calamagrostis rubescens							
1	4/5	2/5	---	0/5	---	---	---
2	2/3	4/5	4/5	0/5	0/2	---	---
3	---	0/1	2/5	---	---	---	---
4	2/2	4/5	---	---	---	---	---
Stipa richardsoni	5/5	---	---	---	1/2	---	---
Achillea millefolium							
1	---	2/3	0/5	0/5	0/5	---	---
2	---	0/5	0/5	0/5	0/5	---	---
3	3/5	2/5	0/5	0/1	0/1	---	---
4	0/5	0/5	0/5	0/5	0/1	---	---
Galium boreale							
1	1/5	2/5	0/5	0/5	1/5	1/5	0/12
2	4/4	2/4	1/5	2/5	0/5	1/10	0/11
3	5/5	4/5	1/5	0/5	0/1	---	---
4	5/5	0/5	3/5	0/5	0/3	---	---
Antennaria spp.							
1	---	1/5	---	---	---	2/3	---
2	0/1	---	---	1/1	---	---	---
3	4/5	---	0/1	1/1	0/2	---	---

*Dash indicates no samples available on the plot

**Plot replicates

Appendix IV (continued)

Species	Depths (inches) 6	12	24	36	48	60	72
*Pseudotsuga menziesii							
1	0/1	---	---	---	---	---	---
2	0/5	0/3	---	0/2	---	---	---
3	0/1	0/4	0/5	0/2	---	---	---
4	0/1	0/2	---	---	0/2	---	---
Trifolium repens	2/3	---	---	---	---	---	---
Vaccinium caespitosum							
1	5/5	2/5	0/5	0/5	0/5	0/2	---
2	2/5	0/5	0/5	---	0/5	---	0/2
3	3/5	4/5	1/5	1/5	0/5	---	---
4	---	0/3	1/5	2/5	0/5	---	---
Rosa woodsii							
1	1/1	---	1/5	0/2	0/2	---	---
2	2/5	0/5	0/3	0/2	0/3	---	0/3
3	---	1/2	0/1	1/5	2/3	---	---
4	1/1	---	0/5	2/5	---	---	---
Geranium viscosissimum	---	2/2	0/2	---	0/2	---	---

*Seedlings less than 6 inches high

Appendix V. Fraction of plants sampled found to be active above background at ecounit 5.

Species	Depths (inches) 6	12	24	36	48
Arnica cordifolia	10/10	1/5	5/10	0/1	0/1
Chimaphila umbellata	0/10	0/10	0/10	0/10	0/3
Carex spp.	**--	0/10	----	0/1	---
Xerophyllum tenax	5/5	4/5	2/5	0/5	0/2
Arctostaphylos uva-ursi	2/2	0/8	----	0/2	---
Calamagrostis rubescens	3/5	9/10	0/5	0/3	0/1
*Pseudotsuga menziesii	1/8	0/2	0/6	0/6	0/5
Vaccinium membranaceum	5/10	0/10	0/7	0/10	0/6

*Seedlings less than 6 inches high

**Dash indicates no plants available for sampling.

Appendix VI. Counting of plant samples taken outside fenced area.

Species	Counts per 5 minutes
Arnica cordifolia	89*
Carex geyeri	67
Symphoricarpos albus	94
Arctostaphylos uva-ursi	99
Berberis repens	72
Fragaria virginiana	86
Calamagrostis rubescens	83
Achillea millefolium	84
Galium boreale	85
Antennaria spp.	108
Pseudotsuga menziesii	82
Vaccinium caespitosum	77
Rosa woodsii	92
Geranium viscosissimum	92

*Each sample represents a composite of samples taken from several plants.

Appendix VII. Lateral root extension; fraction of plants sampled found to be active above background.

6 inch injection depth

Species	Distance from tubes (cm)					
	10	20	30	50	75	100
Carex geyeri (Cage)	*1/2	3/6	0/3	0/5	0/7	---**
Symphoricarpos albus (Syal)	3/4	2/3	0/3	0/6	0/9	0/3
Arctostaphylos uva-ursi (Aruv)	0/3	1/1	---	0/6	0/3	---
Berberis repens (Bere)	0/3	0/2	0/2	1/6	1/5	---
Fragaria virginiana (Frvi)	---	0/2	---	0/1	0/4	---
Calamagrostis rubescens (Caru)	---	---	---	---	---	---
Achillea millefolium (Acmi)	---	---	---	---	0/2	---
Galium boreale (Gabo)	2/4	---	0/3	0/6	0/6	---
Antennaria spp. (ANTE)	0/1	---	---	---	---	---
Rosa woodsii (Rowo)	---	---	---	---	---	---

**Dash indicates no plants sampled

*Top number - plants active; bottom - plants sampled.

Appendix VII (continued)

12 inch injection depth

Species	Distance from tube (cm) 10	20	30	50	75	100
Cage	1/2	1/3	2/3	1/5	0/3	---
Syal	2/2	0/3	1/3	0/8	0/8	0/4
Aruv	0/1	1/1	1/3	0/5	0/6	---
Bere	1/3	0/3	1/2	1/9	0/5	---
Frvi	---	---	0/2	---	---	---
Caru	---	---	1/3	0/3	0/4	---
Acme	---	---	---	---	---	---
Gabo	0/1	1/2	1/1	0/2	0/4	---
ANTE	---	---	---	---	---	---
Rowo	---	---	---	1/3	0/2	---

24 inch injection depth

Cage	0/3	0/3	0/3	0/5	0/5	---
Syal	3/3	2/3	1/3	1/5	1/4	0/5
Aruv	0/2	1/2	0/2	0/5	---	---
Bere	---	1/2	---	0/2	0/2	---
Frvi	---	---	---	---	---	---
Caru	---	---	---	0/1	---	---
Acmi	---	---	---	---	---	---
Gabo	---	---	---	---	---	---
ANTE	---	---	---	---	---	---
Rowo	0/1	0/1	---	---	---	---

Appendix VII (continued)

36 inch injection depth

Species	Distance from tube (cm)					
	10	20	30	50	75	100
Cage	0/3	0/3	0/3	0/5	0/5	---
Syal	0/3	0/3	0/3	0/5	0/5	0/5
Aruv	---	---	---	---	---	---
Bere	---	0/1	---	---	---	---
Frvi	0/3	0/3	0/2	0/3	0/1	---
Caru	---	---	0/1	---	---	---
Acmi	---	0/2	---	---	---	---
Gabo	---	---	---	0/2	---	---
ANTE	---	---	---	---	---	---
Rowo	---	0/1	---	---	---	---

48 inch injection depth

Cage	0/1	0/2	0/3	1/5	0/4	---
Syal	1/3	0/3	0/3	0/3	1/5	0/3
Aruv	---	---	---	---	---	---
Bere	0/2	0/2	1/1	1/4	---	---
Frvi	0/3	0/1	0/3	0/4	0/2	---
Caru	---	---	---	---	---	---
Acmi	---	---	---	---	---	---
Gabo	0/1	0/1	0/2	0/1	---	---
ANTE	---	---	---	---	---	---
Rowo	0/3	---	0/1	---	0/1	---