# Translocation of $^{14}C$ in ponderosa pine seedlings

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The movement of $^{14}C$ from the old needles to the roots, and later to the new needles, was measured in 2-year-old ponderosa pine seedlings. The seedlings were in one of three growth stages at the time of the feeding of $^{14}CO_2$: 9 days before spring bud break with no root activity; 7 days before spring bud break with high root activity; and 7 days after spring bud break with moderate root activity. The form of the curves of count rate plotted against time are similar for a given plant part. Immediately after being fed $^{14}CO_2$, year-old needles had high $^{14}C$ count rates which dropped rapidly, leveled out within 7 to 10 days, and reached a steady state residual count rate after 10 to 20 days. The decrease in count rate from the old needles was followed by an increase in count rate by the root tip and (or) the new needles. The count rate of the root tips increased from background to a peak within 3 to 7 days, then decreased for the duration of the study. The details and timing of movement of $^{14}C$ to and from plant parts was a function of the growth state at the time of $^{14}CO_2$ feeding.

## Introduction

Several investigators have demonstrated the value of exposing pine trees to $^{14}CO_2$ to follow the translocation of photosynthates. Shiroya et al. (3) followed the translocation of $^{14}C$ in several molecular fractions in Pinus strobus seedlings from early spring through late fall. They measured translocation as the amount of $^{14}C$ recovered from the roots at the end of a 7-h period. Plants were grown in open seedbeds under two light conditions. Once a month two plants were removed from the nursery and fed $^{14}CO_2$ in the laboratory. In an extension of this study, Ursino et al. (4) observed the distribution of $^{14}C$ between Pinus strobus roots, old shoots, and new shoots. They also determined the amount of $^{14}C$ lost from the plants at about monthly intervals after photoassimilation of $^{14}CO_2$ and measured the partition of the retained $^{14}C$ fraction. In a separate study, Gordon and Larson (2) measured the distribution of $^{14}C$ in the bud, old and new needles, root, and internodes 10 times during the seasonal growth of Pinus resinosa seedlings. The seedlings were pretreated by pruning in fall, placed in a cold frame, and later moved to a green house and subjected to an 18-h photoperiod with diurnal temperatures of $16^{\circ}C$ to $22^{\circ}C$. Ten times during the season, two trees were fed $^{14}CO_2$ through the old needles, and two trees fed through the new needles. After 72 h the trees were harvested for analysis. Balatinecz et al. (1) fed $^{14}CO_2$ to actively growing 8-month-old Pinus banksiana seedlings. Samples of the root, stem, and needles were taken 0, 1, 2, 3, 6, and 12 days after treatment. Preconditioning of the seedlings was not described. In each of these studies, analysis was by total harvest techniques.

This paper reports a study of the movement of current photosynthates in ponderosa pine seedlings Pinus ponderosa Laws.) at different stages of growth in spring after exposure to $^{14}CO_2$.

## Material and Methods

The ponderosa pine seedlings were obtained from the Colorado State Forest Service nursery in Fort Collins in spring 1969. On April 4, 2-year-old seedlings, grown in 5- by 20-cm tar paper tubes and overwintered in a lath house, were transplanted into 15- by 20-cm cans and placed in the laboratory. At that time the terminal buds had not begun to swell, and there was no new root growth. Air temperature in the laboratory was about $21^{\circ}C$. The seedlings were illuminated 18 h daily with two 40-W gro-lux fluorescent and two 25-W incandescent lamps, to give 330 mW cm$^{-2}$ of blue, 1610 mW cm$^{-2}$ of red, and 1000 mW cm$^{-2}$ far-red light at bud height.[1]

Ten days later, on April 14, two more seedlings grown in 15- by 20-cm cans for 1 year and also overwintered outdoors were placed in the laboratory. The terminal buds were dormant, and there was no new root growth. The bottoms of all seedling containers were removed to permit examination and sampling of the roots. The exposed soil and roots were covered with several layers of cotton gauze.

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[1]Light intensity measured with a model IL150, Plant Growth Photometer manufactured by Industrial Light, Inc., Newburyport, Mass. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture of any product or service to the exclusion of others which may be suitable.

On April 16, two comparable-sized seedlings, one each of the April 4 and 14 groups, were exposed to $^{14}CO_2$. Each seedling was in a different growth stage. On the seedling exposed 10 days to warm temperatures and longer photoperiod, the terminal bud had not broken, but was just beginning to swell. The roots, however, were growing, and the root tips showed 3-5 mm of new elongation. On the seedling just brought from the nursery, where night temperatures still were below freezing, the terminal bud was dormant, and there was no new root growth.

To prepare a seedling for exposure to $^{14}CO_2$ a polyethylene sheet was taped over the top of the can and around the seedling stem to exclude $^{14}CO_2$ from the soil and roots. A 10-ml glass vial holding a small piece of litmus paper was then taped to the stem. After 100 µg of sodium bicarbonate, $NaHC^{14}O_3$, in 1.0 ml sterile water at pH 9.5, were added to the vial, a polyethylene bag was placed over the seedling top and sealed to the can with tape. The seedling then was illuminated with four 40-W fluorescent lamps-two cool white and two gro-lux-and two 150-W and one 100-W incandescent lamps to give 750 mW cm-2 of blue, 3520 mW cm-2 of red, and 3220 mW cm-2 of far-red light at bud height.

To release $^{14}CO_2$, 4 ml of 2 $N$ $H_2SO_4$ was added to the vial by using a hypodermic needle. The acidity of the solution was assured by the color change of the litmus paper. The needle hole in the bag was sealed with tape. The specific activity of the $^{14}C$ was 10 µcuries/100 µg $NaHCO_3$.

For the two seedlings fed $^{14}CO_2$ on April 16, the bags were removed after 8 h. Gordon and Larson (2) found that Pinus resinosa seedlings fed 23 µcurie $^{14}CO_2$ for 2 h

absorbed more than 90% of the $^{14}CO_2$. Ursino et al. (4) found that P. strobus seedlings fed 14 µcurie $^{14}CO_2$ photoassimilated 99% of it during the first hour. Ursino et al. (4) also found that assimilation of 14 µcurie of $^{14}CO_2$ caused no discernible damage in the development of **P. strobus** seedlings. I thus assumed that the assimilation of 10 µcurie of $^{14}CO_2$ by the larger ponderosa seedlings would not affect their growth or translocation,

To check that essentially all the $^{14}CO_2$ was absorbed by the seedlings, the vials, the condensate in the bags, air in the bags, and the bags themselves were examined for residual radioactivity. Only an insignificant trace was found in the air in the bag. No activity was found on the bag or condensate. A small residual activity was found in solution in the vial.

After exposure to $^{14}CO_2$, tissue samples were taken from the radioactively tagged seedlings. At selected intervals, 2-3 cm of root tip and one fascicle each of the year-old needles and the new needles (when present) were cut with surgical scissors from each seedling and immediately placed in a 10-ml plastic vial which was then capped. Within 3 days each sample was removed from the vial, cut into 1 mm lengths, spread evenly on a 2.54-cm diameter aluminum planchet, and counted 0.8 cm from a shielded thin end window GM counter with a window thickness of 1.4 mg cm-2 and a window diameter of 2.7 cm. The values plotted in Figs. 1, 2, and 3 are the means of ten 1-min counts. To evaluate the possible loss of $^{14}C$ by respiration of the samples during the storage period, 10 samples of each tissue type was counted immediately after removal from the seedling and again after a 3-day storage period in the plastic vials. No significant change in count rate was observed. Self-
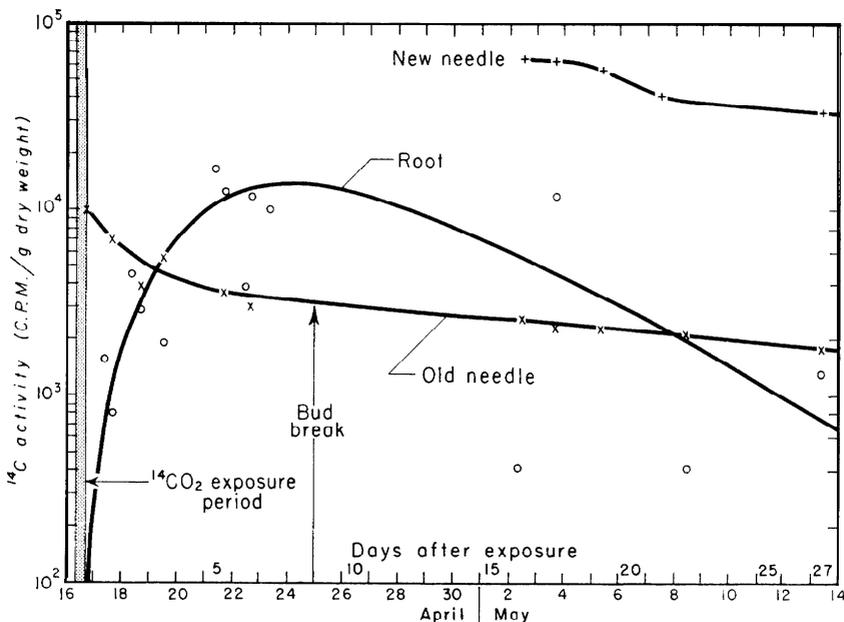


FIG. 1. Time distribution of $^{14}C$ in the old needles, root tips, and new needles of the seedling conditioned in the lath house (seedling 1). The roots were not elongating and the terminal bud was dormant when the shoot was exposed to $^{14}CO_2$.

absorption of the soft p- by the plant tissue and the counting geometry of the individual particles were different with each sample. However, samples within each tissue type were found to yield similar results as long as the tissue was evenly distributed on the planchet. Direct comparison of count rates between tissue types, however, must be made with great caution. After the samples were counted, they were air-dried 7 days, then weighed to express $^{14}$C concentration as counts per minute per gram dry weight. The dry weights of the samples were about 0.01 g for roots, 0.03 g for old needles, and 0.002-0.006 g for new needles, the amount increasing as the new fascicles expanded.

On May 1-16 days after exposure to $^{14}$CO$_2$-the seedling initially showing root growth was lifted and autoradiographed. The stem was cut just above the soil level, and the roots were washed free of soil and blotted with paper towels. The roots and the shoot were flattened and dried in a plant press for 4 days, then placed on a 20- by 25-cm sheet of Type AA Kodak Industrial X-ray film in the dark for 5 days.

On May 1, a comparable size seedling, brought from the lath house on April 14 and conditioned for 15 days in the laboratory, was exposed to $^{14}$CO$_2$ for 18 h in light. Bud break had occurred 1 week earlier, the shoots and new needle fascicles were beginning to elongate rapidly, and an estimated 1-2 cm of root elongation had already

occurred. After tagging, tissue samples were cut from the seedling as previously described.

Once assimilated, $^{14}$C can be lost through respiration, removal of plant parts, or root exudation. When the seedling which was autoradiographed was lifted, samples of soil and soil water were counted. The count rate was not significantly greater than background at the 90% level of confidence.

## Results and Discussion

The form of the curves of count rate plotted against time are similar for a given plant part (Figs. 1, 2, 3). The year-old needles showed high $^{14}$C count rates immediately after $^{14}$CO$_2$ exposure, but the count rate dropped rapidly and leveled out within 7 to 10 days. A steady-state residual count rate was found in the old needles after 10 to 20 days. Balatinecz et al. (I) found the radioactivity in jack pine needles decreased for about 3 days, but that in the stem and roots increased. Gordon and Larson (2) reported that
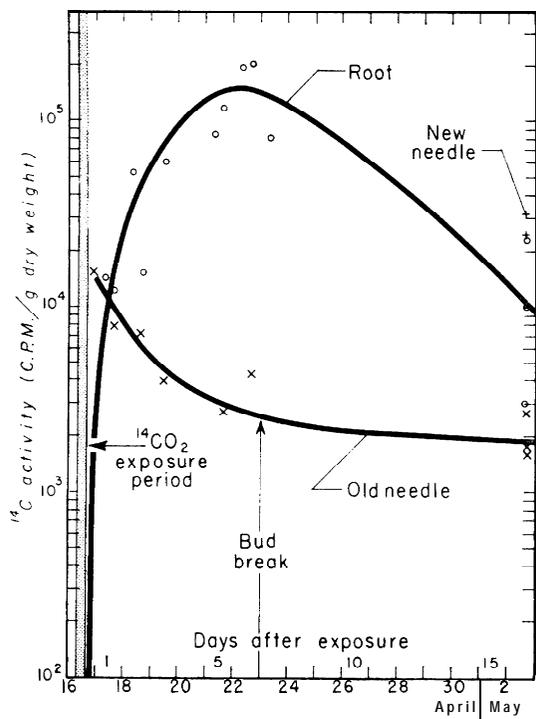


**FIG.** 2. Time distribution of $^{14}$C in the old needles, root tips, and new needles of the seedling conditioned 10 days in the laboratory (seedling 2). The roots were elongating and the terminal bud just beginning to swell when the shoot was exposed to $^{14}$CO$_2$.
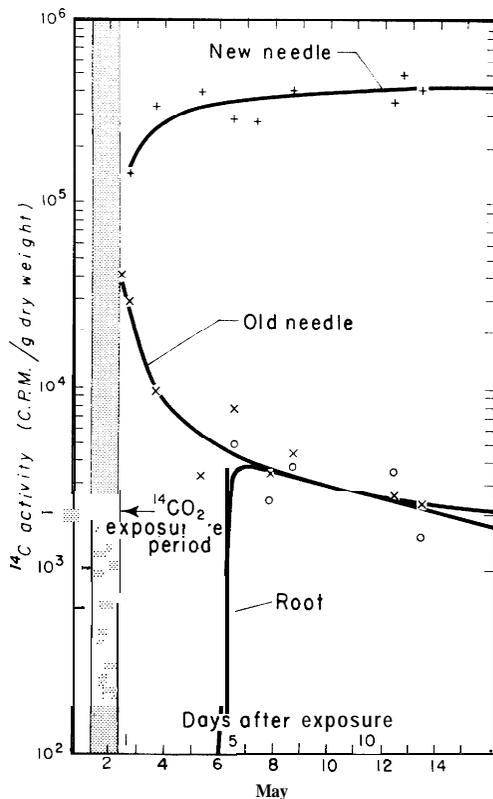


FIG. 3. Time distribution of $^{14}$C in the old needles, root tips, and new needles of the seedling conditioned 17 days in the laboratory (seedling 3). The shoot was exposed to $^{14}$CO$_2$ 1 week after bud break, when both the shoot and roots were elongating.

loss of $^{14}C$ from old needles was primarily the result of translocation rather than respiration.

My results also showed that a decrease in count rate from the old needles corresponds to an increase in count rate by the root and (or) the new needles. For seedling l-bud and roots dormant when exposed-15 days after exposure, the old needles showed 25% of their initial count rate (Fig. 1). For seedling 2-roots elongating, bud swelling when exposed-the old needles showed 14% of their initial count rate after 15 days (Fig. 2). In seedling 3-exposed 7 days after bud break when the shoots were expanding rapidly-12 days after exposure, the old needles showed only 5% of their initial count rate (Fig. 3). On the other hand, the old needles of seedling 1, 27 days after treatment, still retained 19% of their initial count rate. This condition might be explained by the demand for photosynthate in a tree before bud break when such a demand is much less than during active shoot elongation. More of the tagged photosynthate produced in seedlings I and 2 was incorporated into the old needle under low photosynthate demand than in seedling 3 when the demand for export was high.

The buildup of radioactivity of the root tips of seedlings treated before bud break differed from that of the seedling tagged after bud break. In seedlings 1 and 2, treated before bud break, no count rate above background was found in the root tips when the bags were removed, but a substantial count rate was found the next morning, about 24 h after treatment was started. In seedling 3, treated after bud break, no count rate significantly greater than background was found in the root tip until 4 days after feeding. In seedling 2 the count rate of the root tips exceeded the initial count rate of the needles within the first 24 h. This condition suggested a buildup of $^{14}C$-tagged materials in the new, rapidly elongating-root tips. In seedling 1, where the roots appeared to be dormant, about 3 days lapsed before the count rate of the root tips exceeded that of the old needles. In seedling 3, when the new needles were growing rapidly and the growth rate of the root had decreased, the count rate of the root tips about equaled, but did not substantially exceed that of the old needles.

Bud break occurred on about April 25 for seedling 1 and April 23 for seedling 2. This corresponds closely to the beginning of decrease in count rate of the root tips. My data suggest that before bud break, the old needles exported $^{14}C$ tagged materials to the roots. This pattern is similar to that observed by others (1, 2, and 3). The root, in turn, began to export $^{14}C$ substances to the new shoot just before bud break and continued exportation as the new shoot developed.

Ursino et al. (4) showed that current photosynthate is translocated to the developing shoot from the old needles. The same appears to be true in ponderosa seedlings after bud break (Fig. 3). Thus a rapid decrease in the count rate of the old needles was apparently associated with a corresponding increase in the count rate of the new needles. However, both the new and old needles of seedling 3 were exposed to $^{14}CO_2$, and the initial count rate of the new needles was higher than that of the old needles.

In seedlings 1 and 2 the maximum count rate of the root substantially exceeded that of the old needles, whereas in seedling 3 the count rate of the root about equalled that of the old needles. Apparently, when the new shoots are just expanding the current photosynthate from the old needles is translocated to the new shoots, and translocation to the roots is essentially terminated. Gordon and Larson (2) found that translocation of current photosynthate from the old needles to the roots of Pinus resinosa was high just before bud break, but decreased when the new shoot began to expand. Translocation to the root resumed when photosynthesis in the nearly expanded new needles exceeded their growth needs, and photosynthate in the shoot became available for translocation.

One small branch of seedling 3 containing seven l-year-old needles was excluded from the $^{14}CO_2$ atmosphere. Samples of these untreated needles were taken at periodic intervals. At no time during the 13-day sampling period did the count rate of these untreated needles significantly exceed background.

The autoradiograph brought out several points of interest. By far the most radioactive part of the shoot was the bundle sheath material of the new needles. Next most active were the tip of the new needle, the bottom of the new needle, and the middle of the new needle. The old needle had a low film exposure. The old needle bundle sheath and stem were essentially at background. The root showed variable dis-

tribution of activity. Some of the variation was due to self-absorption by the more woody portions of the root, but the greatest activity was concentrated in the elongating root tips.

### Acknowledgments

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