AUTORADIOGRAPHY AS AN AID IN DETERMINING THE GROSS ABSORPTION AND UTILIZATION OF FOLIAR APPLIED NUTRIENTS[1]

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(WITH THREE FIGURES)

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Favorable growth responses have recently been obtained with some phosphorus compounds applied as foliar sprays to vegetable crops, the objective being to supplement the usual supply of phosphorus absorbed by the roots (3). Preliminary studies utilizing a 25 millimolar solution of phosphoric acid enriched with small quantities of radioactive phosphorus indicated that the nutrient was absorbed by the leaves of a wide variety of crops and within a few hours translocated to all plant parts, particularly to meristematic regions.

A technique for making autoradiograms relating to root absorption and subsequent distribution of phosphorus in tomato fruit and leaves at various stages of development has been outlined (1). Suggestions for improving the quality of autoradiograms of biological materials have been described by BOYD (2). As a means of demonstrating the rapidity of absorption, translocation, and selective utilization of foliar applied nutrients, autoradiography offers a unique approach. The effects of chemical agents and variations in the external environment on nutrient absorption can readily be demonstrated. Autoradiograms provide one of the best means of obtaining indications of relative metabolic activities of various plant tissues since their accumulative capacities for many of the foliar applied nutrients is clearly revealed and located. Herein is presented a method of simultaneously preparing a large number of autoradiograms of entire plants, such that the intensity of the images is within limits proportional to the concentration of the radioactive material in the tissues derived from various applications of radio-phosphorus. Autoradiograms are included which illustrate some results obtained.

Preparation of autoradiograms

Bean, corn, and transplanted tomato seedlings were grown in four-inch clay pots of sand and supplied with a minus phosphorus nutrient solution. Reserves of phosphorus in the seeds of bean and corn and in the transplanted tomato plants were in most instances sufficient for seedling growth. When of the appropriate size to occupy an area of contact film having dimensions of 8×10 inches, the foliage or selected parts thereof were treated by momentarily dipping into a 25 millimolar solution of *o*-phosphoric acid

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which assayed 1 micro-curie of P^{32} per milliliter. In some instances specific quantities of the solution were applied to certain vegetative tissues or, for contrast, diluted with distilled water and added to the root cultures. However, for comparable expression of results the above ratio of P^{31} and P^{32} was the same in all treating solutions. Dreft was added as a wetting agent at the rate of 0.2%.

At various time intervals following treatment, entire plants were harvested, and the roots carefully removed and washed free of sand. An attempt was made to remove residual and adsorbed radio-phosphorus on the treated surfaces by successive washing in 25 millimolar solutions of *o*-phosphoric acid and distilled water, respectively, until the washing solutions gave no additional count above background. Excess water was taken up with blotting paper. The entire plant was then placed between 8 × 10 inch sheets of botanical pressing paper. The various sheets of paper containing the plant specimens were then layered between 8 × 10 inch masonite boards and previously heated steel plates which were one-fourth or one-half inch in thickness. The layered pile of plant material, pressing paper, masonite boards, and steel plates was placed under several 250 watt infrared heat lamps for rapid drying. Several one-half inch thick 8 × 10 steel plates were placed on top of the layered stack of plant specimens to facilitate pressing during drying. The pressure of the plates during drying resulted in smooth plant surfaces essential for uniform film contact. The entire interval between harvesting and final drying of the plant tissue was no more than one hour.

Following drying, the plant specimens were allowed to cool and then carefully lifted from the original pressing paper and placed on new sheets. Removal of the plant from the original pressing paper was necessary to avoid smears on the autoradiograms induced by plant juices which may be exuded during the pressing-drying process. Because of the rapidity of the drying process (one hour), movement of the nutrient within the plant during processing was minimized. The new paper with the mounted specimen was then covered with a protective sheet of thin pliofilm held firmly against the plant with Scotch tape. This served to keep the sample in place, preserve it for future reference, and prevent scratching of the photographic film which was placed against it. In order to assure uniform contact with the emulsion, a second sheet of botanical specimen paper was placed on top of the film and the entire preparation mounted between two masonite boards. A special light-proof wooden (white pine) box was constructed to accommodate a number of such units. This exposure box was provided with an adjustable inner steel plate such that, when the preparations were stacked inside with one-half inch steel plates between each unit for shielding and pressure, it could be forced down on the layered stack of specimens by turning a vise handle on the box cover. The film used was 8 × 10 inch Kodak No-Screen X-Ray, which was exposed for five to seven days and developed for seven minutes in X-Ray developer. No fogging of the emulsion resulting from the possible presence of peroxides of terpenes in the wood of the exposure box was evident.

Results

Figures 1, 2, and 3 are illustrative of some of the results obtained. The intensity of the autoradiograms of the plants shown are comparable and representative of the relative quantities of the radio-phosphorus absorbed and mobilized in various tissues, since they were all prepared simultaneously by the methods outlined above. Because of apparent differences in

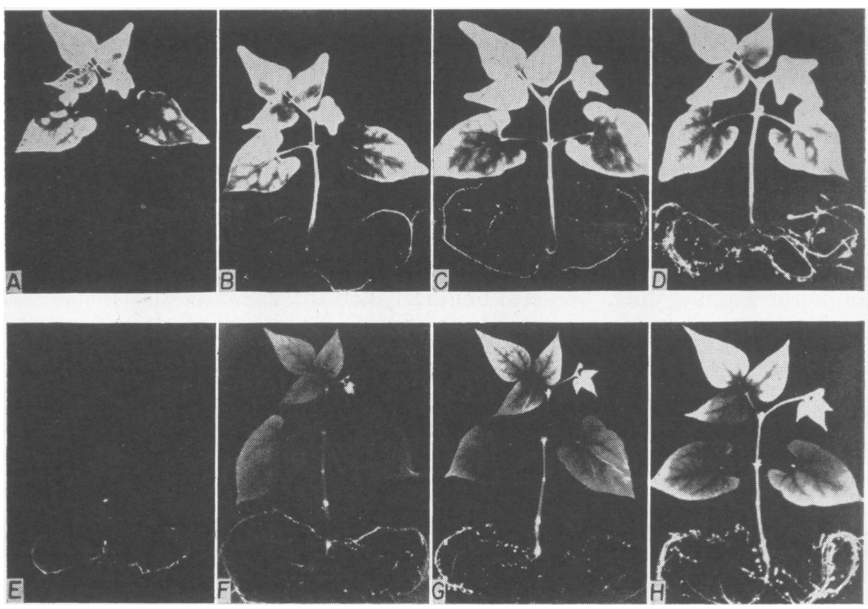

FIG. 1. Autoradiograms of bean plants demonstrating foliar (A to D) and root (E to H) absorption of radioactive phosphorus. From left to right, the autoradiograms were made from plants harvested two, six, 24, and 48 hours following treatment. The apparent distribution of radio-phosphorus in the leaves may not necessarily indicate its precise histological location before processing. Lack of continuity in the roots results from minor breakages and the higher concentrations in the root tips.

rates of leaf absorption and utilization of the radio-phosphorus, some of the autoradiograms, especially those in fig 2, were necessarily over-exposed.

In figure 1, the relative utilization of foliar (A to D) and root (E to H) sources of applied radioactive phosphorus in beans with the progression of time is illustrated. From left to right, the autoradiograms represent plants harvested at two, six, 24, and 48 hours subsequent to treatment. With the foliar applications approximately 0.5 micro-curie of P^{32} was applied to the leaf system of any given bean plant. Root applications consisted of five micro-curies in 100 milliliters of distilled water supplied to each plant grown in a minus phosphate sand culture in a four inch pot. Thus, the specific activity of the phosphate upon addition to the sand cultures was not changed. Approximately 10 times as much radio-phosphorus was applied per plant in the root applications. The relatively greater efficiency of foliar absorption, on the basis of the amounts applied, is suggested. It can also be noted that insofar as time is concerned, the translocation of the nutrient from the leaves to the roots appears to be as rapid as from the roots to the leaves with the concentrations used. This has also been demonstrated by tissue counts and time activity curves using similar plant material, and will

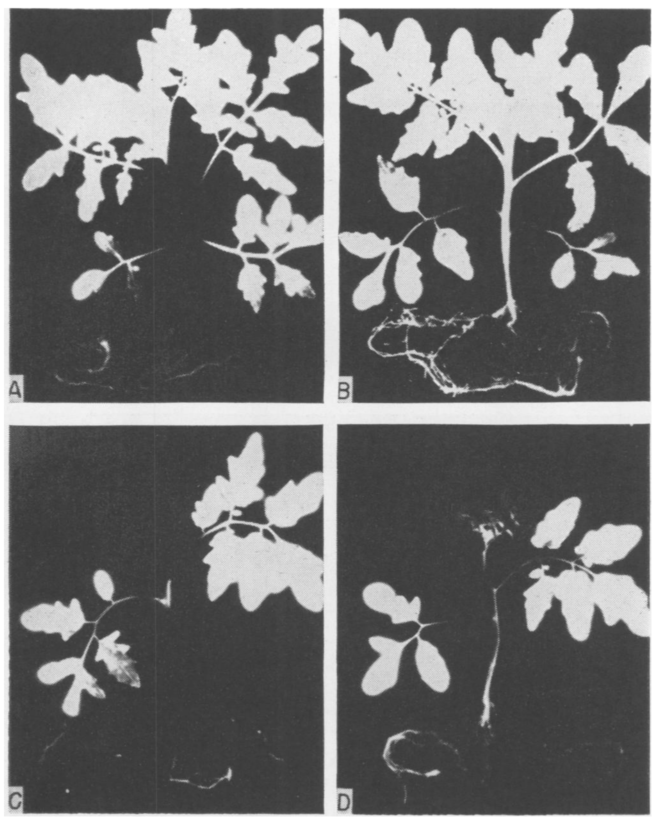

FIG. 2. Utilization of foliar applied radioactive phosphorus in the tomato plant as revealed by autoradiograms. In A and B the entire foliage was momentarily dipped in the radioactive phosphorus and the plants harvested after two and 24 hours, respectively. In C and D only the second and third true leaves were treated and the plants similarly harvested after two and 24 hours, respectively.

be published elsewhere. In all autoradiograms the light portions in the plant tissues designate the presence of absorbed radio-phosphorus. Residual and adsorbed phosphorus was removed before the plants were prepared for film exposure. It can be noted in the autoradiograms of figure 1 A and B, taken of plants harvested two and six hours after treatment, that the younger leaves show the greatest concentration of the radio-phosphorus. This suggests that not only do the young meristematic tissues most readily utilize available phosphorus but that they are most efficient in its absorption. The greater concentration of phosphorus in the young leaves, lateral buds, nodes, root tips, and root nodules follows a similar pattern whether the nutrient is supplied to the foliage or the roots. The gross distribution of radio-phosphorus in the leaves and the conductive tissues therein apparently depends on the age of the leaves, the time following treatment that the autoradiograms were prepared and whether leaf or root applications were employed.

It is clearly demonstrated in the autoradiograms of the tomato plants of figure 2 that foliar applied phosphorus is selectively mobilized by certain

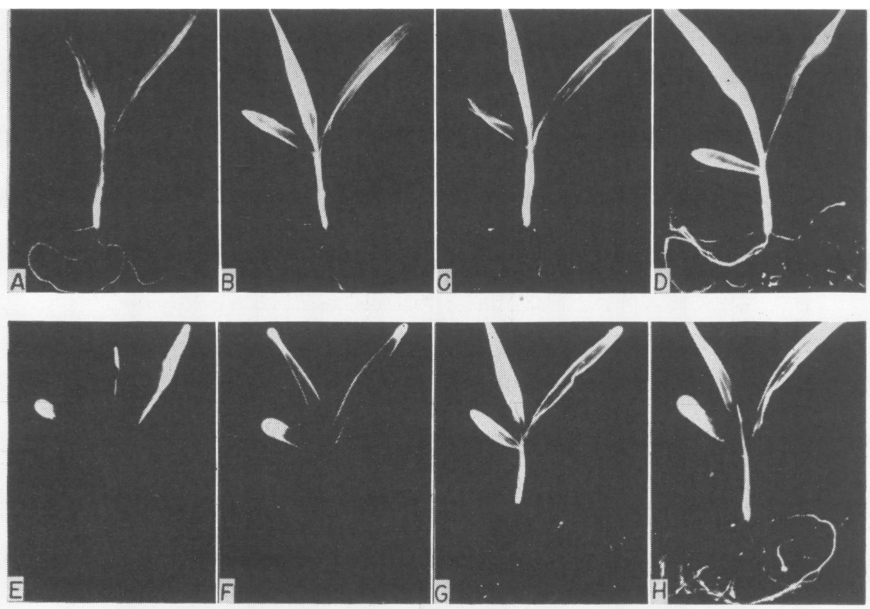

FIG. 3. Autoradiograms of corn plants showing the effect of foliar placement on the absorption by the leaves of radioactive phosphorus. In A to D, the nutrient was placed in the funnel formed by the leaf sheaths of the meristematic leaves of the growing tip. The autoradiograms E to H represent plants whereon only the outer portions of the leaves were treated. From left to right the plants were harvested in both groups at two, six, 24, and 48 hours subsequent to treatment.

tissues. In A and B all the leaves were momentarily dipped into the active solution while with C and D only the second and third true leaves, counting from the bottom of the plants, were treated. The two autoradiograms on the left (A and C) were taken from plants harvested two hours after treatment while those on the right (B and D) represent plants harvested 24 hours subsequent to receiving the radioactive phosphorus. In contrast to the bean plants, it can be noted in the tomato that within two hours of treatment, the phosphorus was absorbed by the leaves and translocated to the roots. Movement to the roots was more rapid than to the vegetative growing tips as is demonstrated in C. It is of further interest to note that after 24 hours (D), the foliar applied radio-phosphorus had accumulated in the root crown, and in the vegetative-growing tip and young leaves, with none apparently moving into the non-treated mature leaves.

The effect of foliar placement of radio-phosphorus on its rapidity of absorption by the leaves of the corn plant is illustrated in figure 3. Autoradiograms A and D were taken of plants harvested at two, six, 24, and 48 hours subsequent to the application of one-fourth micro-curie of P^{32} in the funnel of the growing tip formed by the leaf sheaths, while autoradiograms E and H were developed from plants harvested at the same intervals of time but with only the expanded outer portions of the leaves dipped in the usual solution assaying 1 micro-curie P^{32} per milliliter. The funnel formed by the young leaf sheaths in the center of the growing corn plant consists of meristematic leaf tissue, which provides an advantageous location for applying nutrient solutions. As can be seen in figure 3 A, translocation to the roots and to non-treated leaf portions occurs within two hours after treatment if the radioactive phosphorus is applied in the natural funnel formed by the meristematic leaf sheaths.

Summary

Autoradiography offers a means of obtaining visual records of the gross absorption, translocation and selective mobilization of foliar applied nutrients.

A method of simultaneously preparing a large number of autoradiograms by exposure of 8 × 10 inch Kodak No-Screen X-Ray film to properly prepared plant specimens is described.

Autoradiograms of bean, corn, and tomato plants demonstrate that leaves are efficient organs for absorption of radio-phosphorus. Subsequent distribution in the bean plant of the foliar applied radionuclide is comparable to that taken up by roots. The relatively greater nutrient absorption efficiency of the younger expanding leaves is suggested.

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