HILGARDIA

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOLUME 28 DECEMBER, 1958 NUMBER 6

AUTORADIOGRAPHIC METHOD FOR STUDYING ABSORPTION AND TRANSLOCATION OF HERBICIDES USING C^{14}-LABELED COMPOUNDS

S. YAMAGUCHI and A. S. CRAFTS

UNIVERSITY OF CALIFORNIA · BERKELEY, CALIFORNIA

Various techniques and methods evolved for studying absorption and translocation of herbicides using carbon^{14}-labeled compounds are presented. Advantages of the counting method and of the autoradiographic method are compared, and aspects of plant physiology related to absorption and translocation are discussed. The importance of the autoradiographic method for routine study is emphasized, and complete details of this method are described: choice of plants; greenhouse culture of plants; the specific activity of the tracer; the stock solutions; the treatment solutions; application of the solutions; the number of plants needed for an experiment; treatment time; freeze-killing and freeze-drying; oven drying; open-air drying; autoradiographing; filing of the autoradiographs; and copying of the autoradiographs.

Research and method development reported in this paper was made possible by A. E. C. Contract AT(11-1)-34 Project No. 9.

H I L G A R D I A

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOL. 28 DECEMBER, 1958 No. 6

AUTORADIOGRAPHIC METHOD FOR STUDYING ABSORPTION AND TRANSLOCATION OF HERBICIDES USING C^{14}-LABELED COMPOUNDS[1]

S. YAMAGUCHI and A. S. CRAFTS[2]

INTRODUCTION

WITH THE ADVENT of carbon^{14}-labeled compounds, autoradiographic and counting methods have considerably simplified and broadened some aspects of research on uptake and distribution of herbicides. Formerly, translocation of 2,4-D was tested by some type of growth response, such as bean bending (Day, 1952) and cotton leaf deformity (Clor, 1951). In a similar way, translocation of aminotriazole might be tested on the basis of chlorosis of the new growth. However, any extended research on herbicide action today must include a consideration not just of the uptake and movement of a chemical from the point of application to the site of action, but also of the physiology of the entire plant and especially of the translocation characteristics of the herbicide compound itself. The distribution pattern may be different for each herbicide. Each has its own chemical characteristics which account for its peculiar deviation from a typical pattern of photosynthate distribution.

Now, with the aid of carbon 14[3], physiological concentrations and trace amounts of a herbicide in the tissue can be assayed by the counting method, or they can be autographed[4] for cross-sectional, longitudinal-sectional, or general distribution. A concentration of 2,4-D* which can produce a curvature in the bean epicotyl can also be autographed or counted (fig. 1).[5] A concentration of aminotriazole* which produces a very mild degree of chlorosis in a young bean leaf can be readily autographed. These methods make it feasible to study translocation and distribution of compounds such as maleic hydrazide and monuron, which do not produce characteristic symptoms when applied in trace amounts. They also aid in such difficult situations as tracing the movement of a herbicide in trees, where factors of great distance

[1] Submitted for publication April 16, 1957.

[2] Mr. Yamaguchi is Senior Laboratory Technician in the Department of Botany, Davis; Mr. Crafts is Professor of Botany and Botanist in the Experiment Station, Davis.

[3] Hereafter, for convenience, labeled compounds are designated as 2,4-D*, 2,4,5-T*, etc.

[4] For convenience, the word "autograph" is used synonymously with "autoradiograph" throughout this paper.

[5] J. Pallas, unpublished data, 1956.

and large volume of plant material accentuate all of the difficulties of translocation research.

Carbon 14 has been incorporated into the molecule of a number of herbicide compounds, including aminotriazole, maleic hydrazide, 3-(p-chlorophenyl)-1, 1-dimethylurea (monuron), dalapon, 2,4,5-T, and 2,4-D. The general availability of labeled 2,4-D has greatly extended the studies of its translocation, distribution, and metabolism in the plant (Weintraub *et al.*,

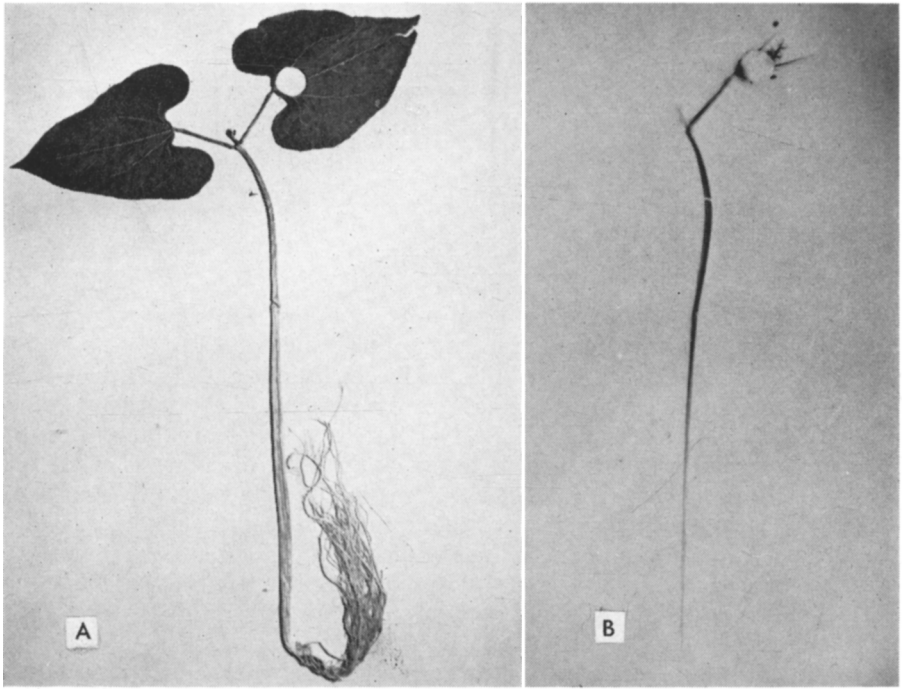

Fig. 1. A red kidney bean plant showing (*A*) the beginning of curvature in the epicotyl and (*B*) the autoradiographic tracing of the chemical two hours after application of 5 μg of 2,4-D-1-C^{14} of 6.03 mc per mM. The treated spot has been removed. (Courtesy James Pallas.)

1952, 1954; Oborn, Lyons, and Timblin, 1954; Fang and Butts, 1954; Crafts, 1956*a*, 1956*b*; Leonard and Crafts, 1956; see, however, Hay and Thimann, 1956).

Carbon 14 has two characteristics which render it particularly amenable to laboratory procedure. One of them is the long half-life of over 5,000 years (Calvin *et al.*, 1949); the other is that its beta rays are readily shielded. The quantities of carbon^{14}-labeled herbicides used in greenhouse experiments require no extraordinary safety measures. Laboratory equipment and procedures are no different from those for ordinary chemicals; however, extra precaution should be taken against spillage or contact with the skin. Because of their cost and toxicity to the plant only micro quantities of the tracers are used. For a consideration of radiation hazards, reference can be made to recent estimates of human radiation tolerances which appeared

in the *Scientific American,* August, 1955, and in summary reports, 1956, of the National Academy of Sciences, "The Biological Effects of Atomic Radiation."

The Counting Method. At present two methods of assessing carbon-14 radioactivity are available. One is the counting method, which employs a

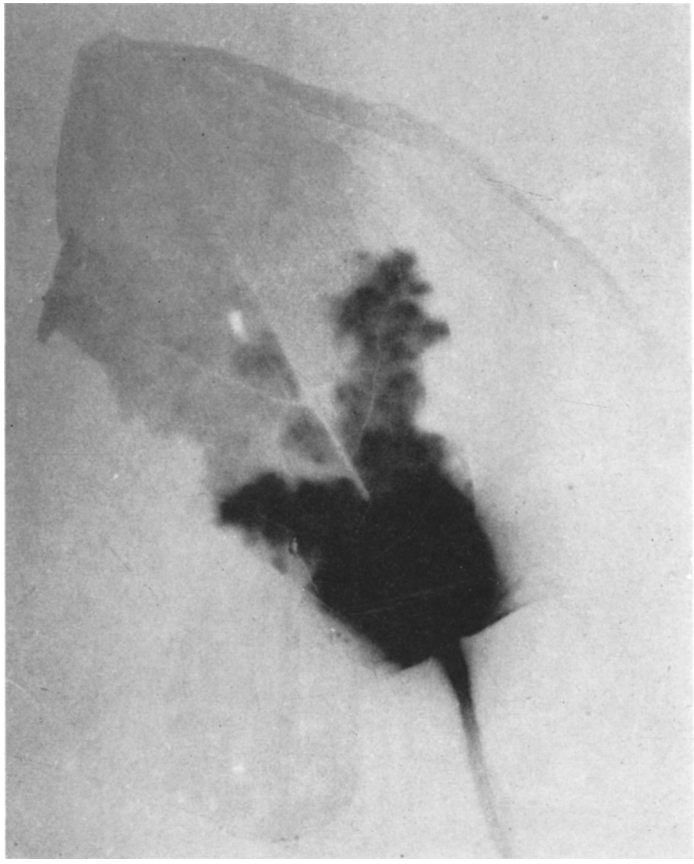

Fig. 2. "Leakage" and "drift" of labeled aminotriazole in the treated leaf of a red kidney bean plant. Treatment consisted of 25μg applied near the base of the leaf blade.

thin-window Geiger-Müller tube or a windowless gas-flow counter. The other is the autoradiographic method, whereby an image is produced directly on X-ray film. The two methods are not closely comparable. In the counting method the plant is cut into parts and each part is counted separately. Usually the parts are organs or fractions thereof. By this method the total amount of the labeled chemical in a bean leaf, for example, can be evaluated. This method is particularly valuable in herbicide absorption studies, for which the autoradiographic method is of less value. It is the only quantitative method for determining the concentration of labeled chemical in a certain amount of tissue. The autoradiographic method gives an image

ranging from a light trace to a black line or mass. Through the range from light to dark a rough approximation of the quantity of tracer present may be made. Beyond the first opaque image no further estimate can be made from the autograph. Counting covers an additional range that may extend far beyond that observable in an autograph.

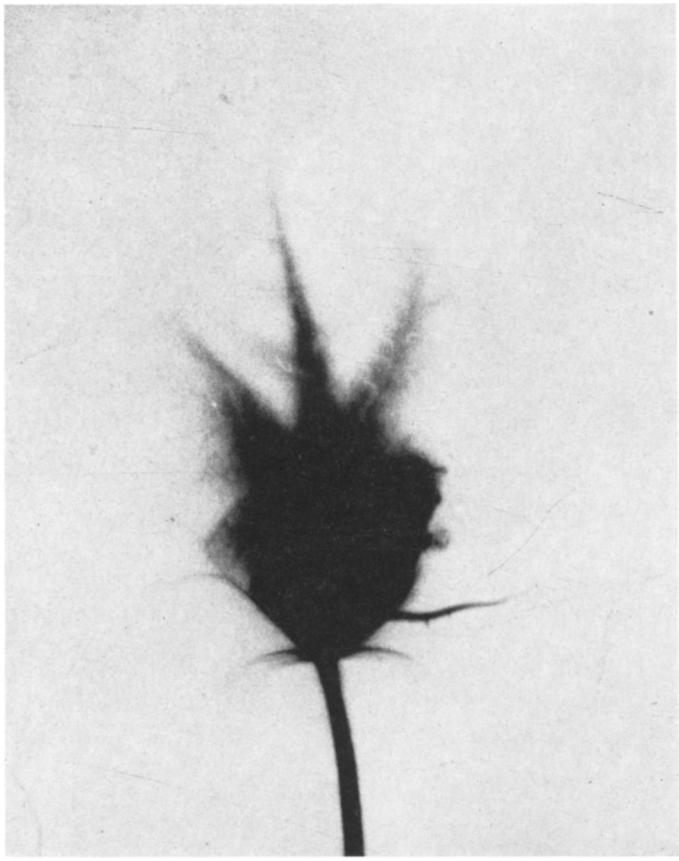

Fig. 3. "Leakage" and "drift" of 2,4-D-1-C^{14} in the treated leaf of a red kidney bean plant. Treatment consisted of 5 μg applied near the base of the leaf blade. (Courtesy James Pallas.)

The Autoradiographic Method. For most preliminary studies of translocation and distribution the autoradiographic method is convenient because the picture of uptake and distribution of the tracer in the entire plant can be observed at a glance on one X-ray film. When the plant material is properly handled, an accurate picture of the extent of distribution of the labeled herbicide is obtained. Often, details such as vascular strands and small veins show up clearly. Within range, the autoradiograph will indicate the difference in concentration of the labeled chemical. Also, such characteristic phenomena as "leakage" and "drift"[6] of a herbicide within the treated leaf

[6] The term "leakage" is used to describe free movement through the tissue where the chemical is applied; "drift" and "drag" refer to movement in the transpirational stream.

(figs. 2 and 3), absorption along the translocation path (fig. 4), accumulation at growing points (fig. 5) and elsewhere, and other phenomena are readily observed.

Along with these advantages of the autoradiographic method there is one important shortcoming. This is the low penetration of carbon-14 beta rays.

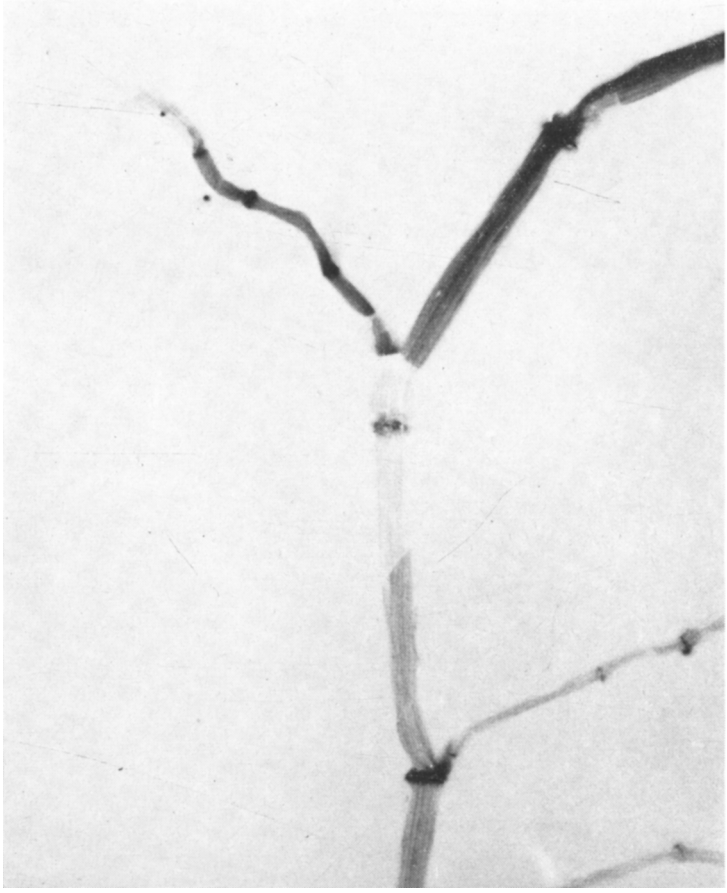

Fig. 4. Absorption of 2,4-D-1-C^{14} along the translocation path in the stem of *Tradescantia fluminensis*. The upper left branch shows lack of accumulation at the growing tip.

Absorption is increased approximately seven times by doubling the thickness of the absorber (Calvin, 1949). Absorption by a single thickness of ordinary cellophane reduces the image intensity only slightly; dried bean leaf, about half; a dried bean stem or Scotch masking tape reduces the image intensity to zero, except when the source is an application spot. In common herbaceous plant stems the tissues peripheral to the vascular tissue are sometimes thick enough to absorb 50 per cent or more of the incident rays. This absorption and resultant loss of image intensity interferes with the detection of the first traces of the labeled chemical translocated into the stem. The use of high activity and maximum exposure time will obviate

most of this difficulty, but only with sacrifice of some of the definition by overexposure in areas behind the front. With large herbaceous and woody plants, the bark has to be peeled and the inner side or the cross sections autographed.

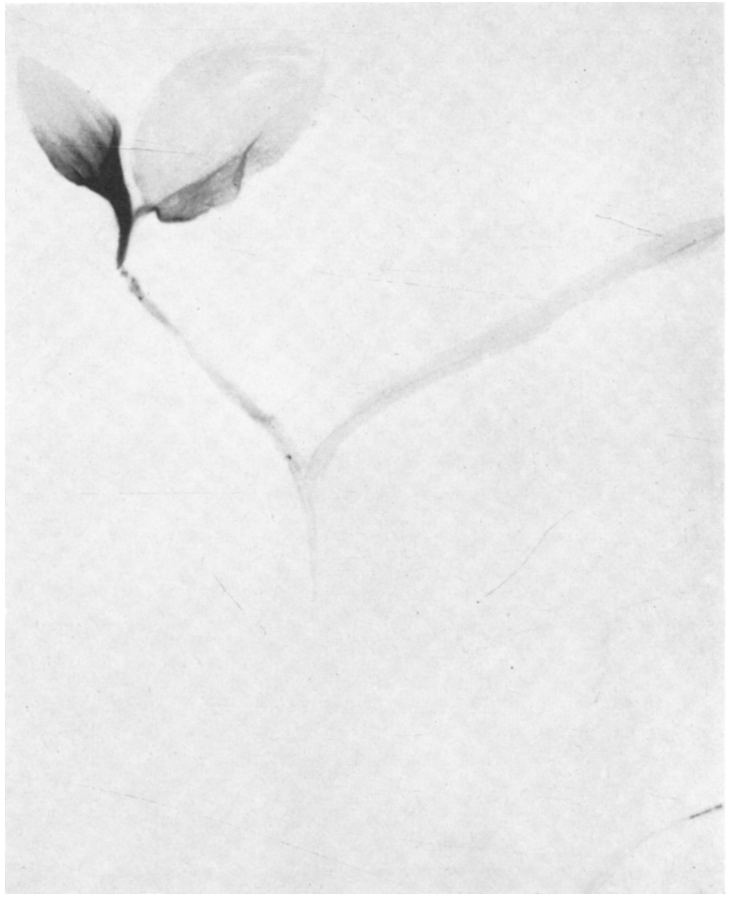

Fig. 5. Accumulation of labeled aminotriazole at the rapidly growing regions of a branch of *Tradescantia fluminensis*. Very little of the chemical is absorbed along the path of translocation.

Autoradiography was used as a major source of information in studies of the relationships of the growth phase of the plant to the amount and direction of translocation of 2,4-D (Crafts, 1956*a*). For routine investigation we have used such plants as beans, cotton, cucumber, wild morning-glory, and *Zebrina*. Also, when the whole plant was too large to handle or too thick to autograph clearly, bark samples and sections of stems of woody plants have been autographed (Leonard and Crafts, 1956).

The autoradiographic method, with modifications, has been adapted to translocation studies not only of herbicides but of insecticides and mineral nutrients as well (Colwell, 1942; Yarwood and Jacobson, 1955). Also, with

the use of special technique and very fine-grained film, micro-autoradiographs have been made of cross sections of plant material (Biddulph, 1956).

In spite of all that has been accomplished with it, this method itself has certain inherent limitations. Graininess of the film is one of them. The other important one is the multidirectional radiation. Even with the closest possible contact of the plant material against the film, there is still a certain lack of definition caused by the thickness of the film and the plant material. The image, instead of being sharply outlined, has a hazy shadow around it. The shadow becomes objectionable with high intensities of radiation, such as occur in the treated area and with overexposure. It is minimized by maintaining close contact between the plant and the film and by using proper exposure time. This method, therefore, is not suited to detailed cytological studies.

Absorption of Foliage-Applied Herbicides. A prime consideration is that leaf surfaces are in general covered by a lipoid layer, the cuticle, which sometimes interferes with satisfactory penetration. Leaves with very little cuticle and large surface area present no problem. They will absorb a fair amount of the applied chemical under various conditions of formulation and application. Other leaves require some understanding of the nature of their cuticle and consequent modification of treatment if adequate absorption is to be achieved.

Much research indicates that the cuticle is extremely variable (Orgell, 1955). Under low-light, high-humidity conditions, the cuticle of some species is thin and quite permeable to water and salts. The cuticle of other species grown under more arid conditions and that of xerophytes may be thicker and practically impervious to such polar compounds. It may, however, be permeable to oils and nonpolar forms of herbicides. Furthermore, the cuticle of some plants seems to be not continuous but interrupted by polar phases that are permeable to water and salts. The fairly ready absorption of compounds like maleic hydrazide under high-humidity conditions would seem to indicate an aqueous route through which compounds may enter plants.

Herbicide formulations are also variable; they may involve oils, emulsions, aqueous solutions, and suspensions. For example, 2,4-D is available and is used as the suspended acid in water; as the emulsifiable acid, applied as an emulsion; as the sodium, ammonium, or a variety of amine salts in water; and as light and heavy esters applied in oil or emulsion form.

It is obviously impossible to explore all of the combinations of the situations described above in any moderate program of research. We have compromised by using such materials as are soluble in 50 per cent alcohol. Examples of compounds dissolved in this medium are 2,4-D, 2,4,5-T, aminotriazole, maleic hydrazide, and monuron. Lower concentrations of alcohol allowed 2,4-D acid and monuron to crystallize out; higher concentrations were avoided because of possible injury to leaves and excessively rapid drying. Tests proved that 50 per cent alcohol produces little or no injury within the time that it normally remains as liquid on the leaves of bean plants. Most of the other plants used are less sensitive to alcohol than are beans.

Early tests proved that the inclusion of a surfactant materially improved absorption of 2,4-D; during preliminary trials Trem 615 was used at a

concentration of 0.1 per cent in many treatment solutions (Crafts, 1956*a*). In 1950 and 1951 several screening tests on surfactants were made, and Nonic 218 was selected as an effective one of low toxicity. Recently Tween-20 at the same concentration has been adopted, mainly because it is used in many laboratories throughout the country in experimental work.[7] From our experience, it seems that surfactants not only enhance wetting by reducing surface tension, but that they also bring about a more intimate contact with the cuticle, may promote penetration of stomates on leaves where these occur, and apparently facilitate penetration of the cuticle by some herbicides, possibly by effecting a physical change of the layer.

Not only the proper formulation and the right surfactant but also a high concentration of the herbicide can facilitate absorption by leaves having thick cuticle. Apparently, with such leaves absorption of some herbicides is not a linear function of concentration. For *Zebrina pendula* there is a minimal concentration for effective penetration of aminotriazole and 2,4-D (Yamaguchi and Crafts, 1956). At concentrations of 500 ppm and lower, 2,4-D has repeatedly failed to penetrate the upper surfaces of the leaves to any extent. Aminotriazole at 1.25 per cent and 2,4-D at 0.25 per cent may be considered minimal concentrations for effective penetration. In the case of *Zebrina* this impermeability may well be a characteristic of the plant species. In the bean leaf there has been no obvious tendency for the absorption of 2,4-D, aminotriazole, or maleic hydrazide to be restricted at lower concentrations.

Temperature is likewise important for satisfactory absorption of the applied chemical. If it is to be applied to the leaves in the form of a solution, temperatures of 80° F or above are preferred. It is well recognized that in the field various herbicides are readily absorbed on warm days. In the greenhouse, too, the phenomenon is a familiar one. A drop of herbicide solution applied near the base of the leaf blade disappears rapidly during hot weather. The entire drop appears to be "sucked in."

Undoubtedly the surfactant qualities of the herbicide, the surfactant additives, and the toxic qualities of the herbicide all take part in making the leaf more permeable to the herbicide solution. If good absorption has occurred, autoradiographs of leaves given a one- or two-hour treatment will often show a drag or drift of the applied chemical from the application spot toward the tip or margin of the leaf, as shown in figure 2. This phenomenon occurs regularly in our greenhouse experiments when droplet applications are made near the base of the leaf blade. It occurs to a lesser extent with 2,4-D than with aminotriazole and maleic hydrazide. It is probably restricted to movement in the transpiration stream, in the xylem, and in the intercellular or cell wall space. The drag occurs as a function of water movement in the leaf on warm days as the applied drop is sucked in. In translocation experiments this rapid absorption is absolutely necessary. Prolonged exposure of the drop on the surface would dry up the solution, and the minimal amount of the labeled chemical necessary for tracing translocation would not be absorbed.

Translocation of Herbicides. Our use of labeled herbicides has involved

[7] O. A. Leonard, personal communication, 1956.

primarily the study of translocation. Extended studies using bean, cotton, cucumber, wild morning-glory, and woody plants have been conducted (Crafts, 1956*a*, 1956*b*; Crafts and Leonard, 1956). An artifact encountered in the early studies has been described (Crafts, 1956*a*; Pallas and Crafts, 1957). The present paper is concerned with a description of methods by which critical results of translocation trials may be obtained. Freeze-drying has been adopted to kill and dry plants in such a way that the autograph obtained from the dried plant represents the distribution of the tracer at the time the plant was killed.

Where uptake by intact roots and general distribution through the xylem are desired, application is made to the culture solution. Where phloem transport is desired, droplet applications to stems and leaves and spray treatments on foliage have been used. Rings of lanolin, silicones, and other retaining materials have been used to confine the treatment to a particular area.

Detailed description of the results of the first three years' studies, during which time methods were modified and improved, is given in the paper cited above. Herein, the methods developed are described for use by others who are interested in employing tracer techniques for translocation studies.

METHODS

Choice of Plants

Both the counting and the autoradiographic methods involve several steps of procedure in the laboratory, and time does not permit large replications or too many gradations of the variable. The type or size of the plant is a less important consideration in the counting method, but it is of prime importance in the autographic method. The size of the plant is considered in terms of ease of mounting on a 10×12-inch sheet of paper, generally in entirety, but in sections when necessary. Small plants of beans, cotton, wild morning-glory, wild mustard, oak seedlings, barley, etc., are readily managed. Bermudagrass is difficult because the leaves are too narrow for easy application of treatment solutions. Elongated plants, such as wild morning-glory, are much more easily handled and studied than rosette plants, which are usually avoided because overlapping of leaves and congested nodes shield the vascular tissues from the film.

Zebrina pendula and *Tradescantia fluminensis* require two weeks to over a month to freeze-dry, depending upon their succulence. Nevertheless, great uniformity of plants can be obtained from cuttings. They are easy to culture in soil or solution without forced aeration. Moreover, stems 2 feet long can be mounted in entirety on 10×12-inch paper.

Large plants that cannot be freeze-killed and freeze-dried in entirety can be sectioned first and then freeze-killed and freeze-dried, or just air-dried if the sections will dry in a few hours. A small amount of radioactive fluid is lost at the cut ends, and care must be taken to keep the exuding fluids from flowing to other parts of the cut surface. The fluids may be blotted off, or, after drying, a thin layer of tissue at the surface may be cut away. After a treatment period of one or more days, radioactivity in the moving

fluids has decreased considerably, and generally very little radioactive contamination or loss will occur upon sectioning.

It is also possible, with considerable time and care, first to freeze-kill a portion of a plant by packing that portion with dry ice, and then to remove a section with a saw or knife. From the time of freeze-killing, such sections must not be allowed to thaw until dry.

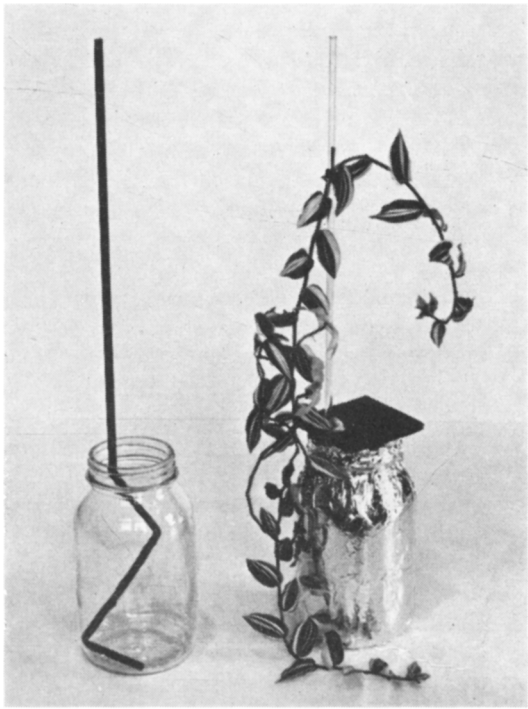

Fig. 6. The quart Mason jar on the left shows a plant support made of glass tubing; on the right, the plant support holds a *Tradescantia* plant in position.

No plant, including trees, should be considered impossible to work with. Special methods for applying the solutions and taking sections or samples can always be devised.

Greenhouse Culture of the Plants

Plants are generally started in sand and transferred to solution culture in quart Mason jars. *Zebrina pendula* and *Tradescantia fluminensis* cuttings are started in tap water. Plants may be grown in soil, but soil is very difficult to remove from the roots, and running water must be used to prevent radioactive contamination through water. Culture solution jars are wrapped with aluminum foil to exclude light. Plants are commonly supported by notched and paraffin-soaked squares of cardboard, by notched cork, or by glass tubing bent so as to be anchored in the jar (fig. 6). Forced aeration of culture solutions is not essential with these species.

To study translocation of chemicals into the roots, a special technique is used because of the possibility of leakage of the chemicals into the culture solution (Clor, 1951). The loss is prevented by spreading the roots on moist filter paper in 6×18-inch glass cylinders and keeping the culture solution approximately 2 inches below the roots. Plants with fine roots are useful for this method. The plants are transferred to the glass cylinders three or four days before treatment.

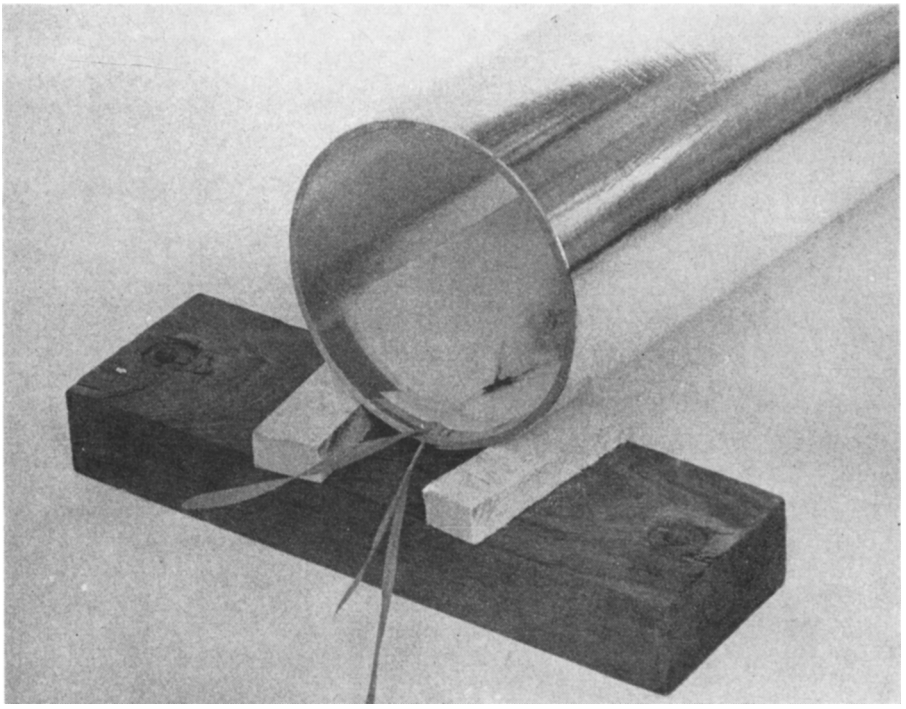

Fig. 7. The roots of a barley plant spread on a filter paper. The plant is held in position with masking tape.

Procedure. Place a 6×16-inch piece of filter paper inside a 6×18-inch glass cylinder, with the upper margin of the paper 1 inch below the lip of the cylinder. Attach the filter paper to the cylinder with masking tape. Pour culture solution into the cylinder, then tilt and slowly roll the cylinder to and fro until the filter paper is entirely moistened and spread evenly against the wall of the cylinder. Prop the jar in such a position that the culture solution is over the filter paper and up to its upper margin. Bring the plant into the cylinder. Spread out its roots in the culture solution over the upper part of the filter paper, allowing some room for root growth below. The lower 4 to 6 inches of paper acts as a wick and is removed later. Hold the base of the plant against the cylinder wall with masking tape. Now slowly raise the cylinder to an upright position, leaving the roots spread evenly over the paper. Place aluminum foil, loosely fitted, across the top of the cylinder to keep out light and draft. The filter paper will need ad-

ditional moisture from day to day. Provide this by tilting the cylinder and pouring the solution in slowly so as not to disturb the roots, or use an atomizer. Also, the frequency of watering can be reduced if more than a single thickness of filter paper is used. Do not spray water onto the roots during the treatment period. (See, however, other details shown in figures 7 to 10.)

Cotton plants have relatively large and stiff roots which do not produce root hairs readily in culture solution, and they are handled somewhat dif-

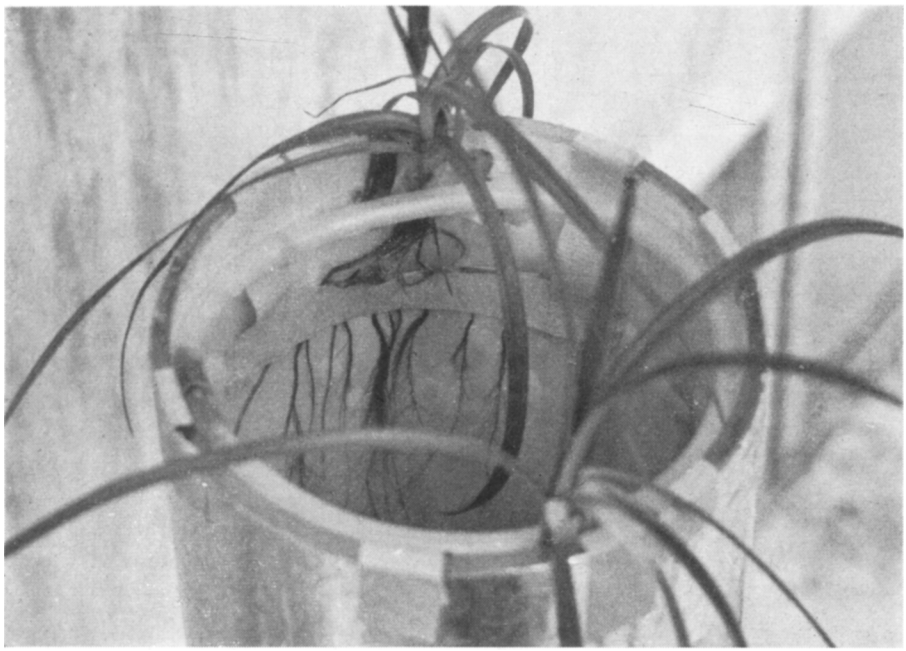

Fig. 8. A ring made of polyethylene tubing supports the crowns of nutgrass plants against the cylinder wall. (Courtesy Otto Andersen.)

ferently. When the roots of the seedlings are 3 or 4 inches long, transfer them to half-strength Hoagland's solution in bottles. Four days before treatment transfer them to the 18-inch glass cylinders, as follows.

Instead of a single sheet of filter paper, use three or four. Instead of placing the plants directly against the cylinder wall, lay them flat against a glass plate $5\frac{3}{8} \times 17\frac{3}{4}$ inches, which will fit into the glass cylinder. Here again, leave an inch of margin at the top of the glass plate. Hold the filter paper up to the margin and tape it down at the margin. Place the glass plate with the filter paper in 2 inches of the culture solution in a shallow tray, propping up the top end of the glass plate above the solution. Place a cotton plant in position on the filter paper and spread out the roots. Tape the base of the plant tightly to the top margin of the glass plate (fig. 9). Gently remove the glass plate with the plant from the tray. Stretch a piece of thin polyethylene material entirely over the roots and wrap it around the edge of the glass plate; holding the ends tight, tape them down on the back side

of the glass plate. Place the glass plate in the glass cylinder, bringing the level of the culture solution 2 inches above the tips of three or four of the longest roots. Fit a piece of aluminum foil over the glass cylinder. (In this manner we have been able to prevent wilting of cotton plants and yet keep most of the roots well above the water level.)

When a labeled chemical leaks from the roots, most of the leakage will be retained on the filter paper. Bacterial growth often becomes excessive

Fig. 9. The use of a glass plate to support the filter paper facilitates the handling of the roots, as compared with the method shown in figure 7.

on the filter paper after several days. If necessary, sterilize the filter paper before use with chlorinated lime and rinse thoroughly. If the roots become so long that the entire plant cannot be mounted on the usual 10×12-inch sheet of paper, use a 10×24-inch sheet. The boards for pressing and autographing must, of course, correspond in size. The sponge rubber and the film, however, are the usual 10×12 inches, with two pieces fitted together. After autographing, the mounts are cut in half, top and bottom, so that mounts and autographs can be filed in the usual manner.

Specific Activity of the Tracer

Some of the labeled herbicide compounds were supplied by the respective chemical companies[8]; 2,4-D* and 2,4,5-T* were purchased from Tracerlab,

[8] The monuron* used in these studies was supplied by E. I. Du Pont de Nemours and Co., the maleic hydrazide* by the Naugatuck Chemical Co., and the aminotriazole* by the American Chemical Paint Co.

Inc. Generally available 2,4-D* and 2,4,5-T* are synthesized to the specific activity of about 1.0 mc per mM; however, on request, 2,4-D* of 5 or even 10 mc per mM may be obtained from Tracerlab, Inc. These higher activities are useful, particularly with regard to these two chemicals, because dilute solutions are effective where a more concentrated solution would cause con-

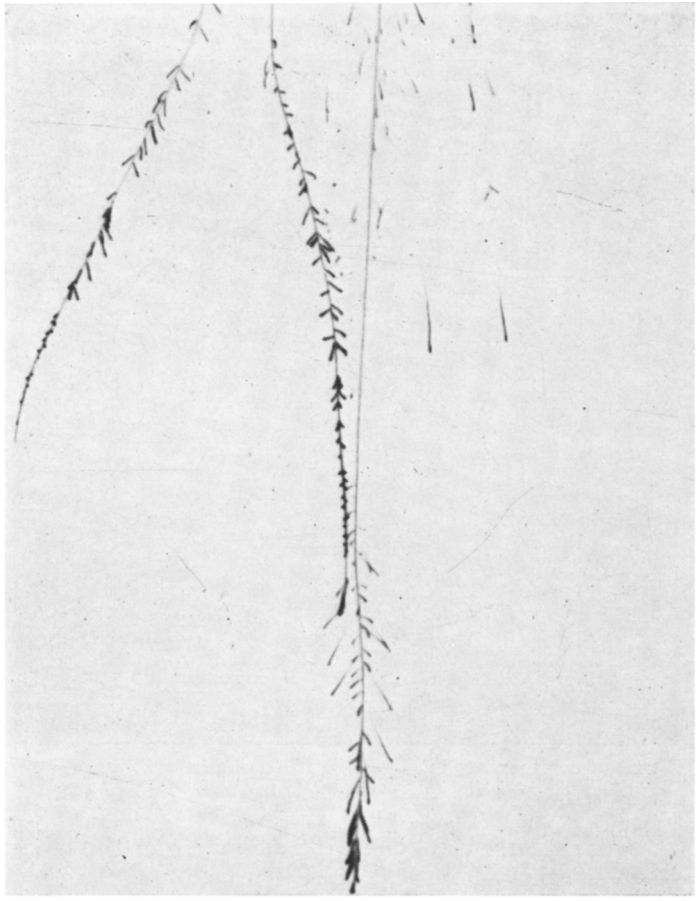

Fig. 10. This autograph illustrates the superb results obtainable when filter paper is used as a medium for root growth. The position of even the finest roots is not disturbed; furthermore, treatment chemicals that would leach out from the roots with the solution-culture method are retained. These are roots of *Cyperus rotundus* (1½ times natural size) after treatment of a leaf with 0.5μc of labeled aminotriazole. (Courtesy Otto Andersen.)

tact injury. Nevertheless, for many plant species, 2,4-D* of the specific activity of 1.0 mc per mM has been perfectly satisfactory. Most of our labeled chemicals were obtained at activities of 1.0 mc per mM or more; the activity of our maleic hydrazide* was 0.5 mc per mM.

In the case of foliar-applied chemicals, the adequacy of the specific activity is determined by the minimal amount of the chemical that carries sufficient radioactivity for assay by the autographic and counting methods. The mini-

mal effective amount of 2,4-D* (1.0 mc per mM), with our drop-application method, ranges from 1.0 to 50 μg (0.0045 to 0.225 μc), depending on the pattern of distribution, the plant species, the size of the specimens, and sometimes on the treatment time in the case of chemicals that are metabolized or lost from the plant body. The amount of radioactivity required ranges from 0.0045 μc or less for tracing translocation in a small barley leaf to 0.50 to 1.00 μc for nutgrass, *Zebrina,* and plants larger than beans in the primary leaf stage. The specific activity of 0.5 to 1.0 mc per mM is therefore adequate under most conditions.

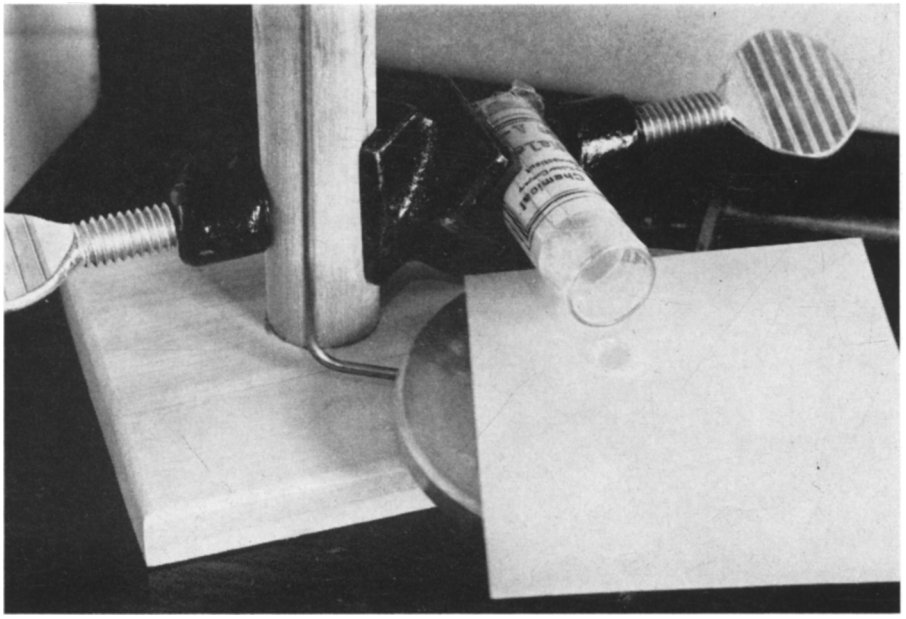

Fig. 11. The arrangement used to weigh labeled chemicals, showing the support used for the original container of the labeled chemical.

The desired number of microcuries is the minimal amount required for the assaying methods; phytotoxicity sets the upper limits of dosage in the case of 2,4-D* and 2,4,5-T*, and cost of the labeled chemicals in the case of others. The only advantage of using greater amounts in the counting method is the shorter counting time. In the autographic method the limitation of the film must also be taken into account. The image intensity in the film is useful within a visible range of differences of about tenfold. Beyond these limits the image is either too light or too dark and difficult to assay. The use of transmitted instead of reflected light to examine the autographs extends the useful range somewhat.

For the reasons given above the autographic method requires a close adjustment of the radioactivity to the exposure time. The radioactivity used in our routine experiments is adjusted to four weeks' exposure time. Maximum use is thus made of small amounts of radioactivity, and phytotoxicity is held to a minimum. Several thousand individual applications may be

expected from 1.0 mc. If the time element is important, larger amounts of radioactivity may be used to reduce the exposure time. Aminotriazole*, maleic hydrazide*, and some others may be so used. 2,4-D* and 2,4,5-T* can also be considered for short time exposure of a few days if a source higher in specific activity is used and if the plant species selected is one of those more tolerant to 2,4-D. Contact injury must be held at a minimum because it prevents movement of the chemical out from the area of treatment.

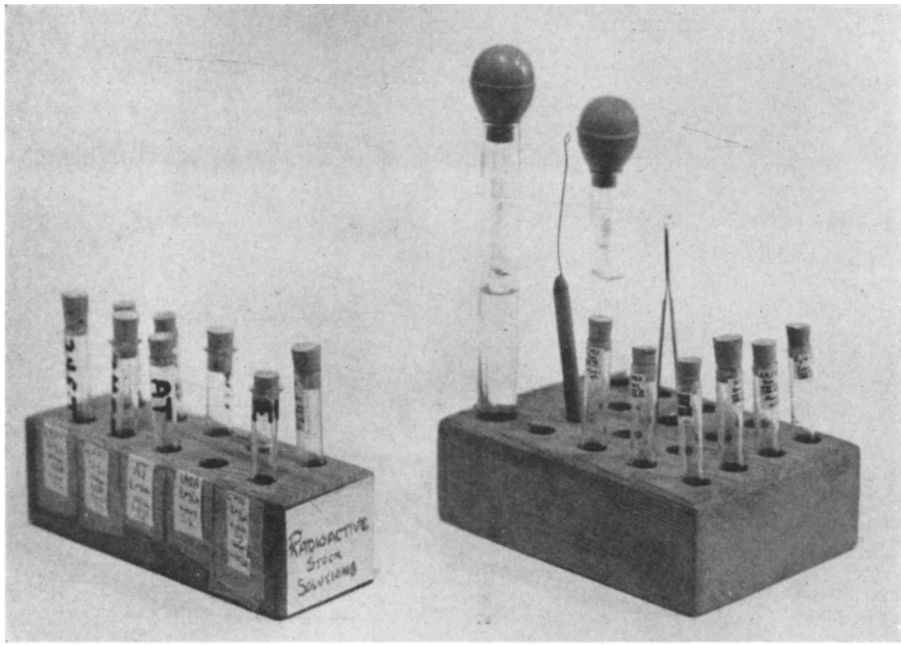

Fig. 12. Convenient arrangements for the radioactive stock solutions (left) and the treatment solutions (right). The larger test tubes contain rinse fluids and micropipettes.

The Stock Solutions

The actual weight of the labeled chemical used in one experiment is usually less than 1 mg. Therefore, for general use, the chemicals are not weighed out each time but, rather, are weighed in relatively large amounts, and stock solutions are made. We prepare these in quantities of 1 to 2 ml, and we find 50 μc per ml a convenient concentration. A fraction of a milligram of the labeled chemical is readily pipetted out from such a stock solution with a micropipette; a small number of microcuries can be taken by aliquot.

Special equipment necessary in the preparation of stock solutions includes micro weighing dishes, a microspatula, a support (fig. 11) for the original container of the labeled chemical so that it can be held rigid and horizontal close above the balance pan, 10×75-mm culture tubes, a wooden test-tube support, forceps, a set of self-filling micropipettes ranging in capacity from 1 to 10 μl, a $\frac{1}{10}$-ml capacity pipette graduated to $\frac{1}{100}$ ml, and a 1-ml volumetric pipette. The micro weighing dishes can be made from fragments of

microscope slide cover slips by cupping them slightly over a well-adjusted flame. Directions follow for preparing a stock solution from 2,4-D* of the specific activity of 1.0 mc per mM.

Procedure. Preparation of 1 ml of stock solution containing 50 μc requires 11.05 mg of 2,4-D*. Placing the vial of the labeled chemical above the balance pan, as shown in figure 11, weigh out between 10 and 20 mg on a micro weighing dish and adjust the volume of the solvent to the actual weight of the chemical instead of trying to weigh out exactly 11.05 mg. In the case of 2,4-D*, acetone, reagent grade, is used as the solvent instead of alcohol because alcohol can react with 2,4-D*. Deliver the calculated amount of acetone, and stopper the container.

Similar steps are followed in the preparation of other stock solutions. Some stock solutions may be alcoholic and others aqueous, depending on the solubility of the compounds. If not objectionable, 50 per cent alcohol is preferred over the aqueous solutions because droplets of the solvent will not cling to the wall of the container nor will the solutes deteriorate with bacterial action. All of our stock solutions are made up to 50 μc per ml in quantities of 1 to 2 ml. The information to go with the stock solutions should include the name of the chemical, its specific activity, its concentration in ppm, the number of microcuries per unit volume of the solution, and the name of the solvent. The stock solutions are arranged in a wooden test-tube support (fig. 12) and stored in the refrigerator.

The Treatment Solutions

Two important aspects of the treatment solution are (1) the amount of the chemical in the treatment dose and (2) the radioactive intensity of the treatment dose. Where penetration and translocation per se are the processes under study and injury should be at a minimum, the tracer should be at the highest specific activity obtainable. As an example, if 2,4-D* is used at a specific activity of 10 mc per mM and this tracer is to be applied at a dosage of 0.05 μc per treatment in 10 μl of solution, the treatment solution should contain 1.105 μg per 10 μl, or 110.5 μg per ml. A convenient stock solution may contain ten times this concentration, or 1.105 mg per ml. Applied at this rate, 2,4-D will have slight but perceptible formative effects on young cotton plants; symptoms may be detectable on bean seedlings; these effects cannot be termed injury, and they do not inhibit uptake and translocation. At ten times this dosage, as will be required for the same radioactivity from a lot with specific activity of 1.0 mc per mM, 2,4-D symptoms on cotton will be drastic and on bean, very prominent. On the other hand, if a chemical produces only minor injuries and formative effects but has relatively low penetrability, the amount of chemical required in the treatment dose will be high, and specific activity of 0.5 mc per mM will be adequate; the same amount of activity, 0.05 μc, will be used per 10 μl of treatment solution, but the concentration of the chemical will be high (22.1 μg).

When comparative tests of a number of labeled compounds are being made and one wants the radioactive intensity and chemical dosage to be uniform, he is limited by the lowest specific activity among the materials being used. For instance, in studies using 2,4-D*, 2,4,5-T*, aminotriazole*, maleic hydra-

zide*, and monuron*, the maleic hydrazide* obtainable had a specific activity of 0.5. Since we wanted the molar dose, as well as the radioactive intensity, to be the same for all, the other chemicals had to be adjusted to this specific activity. Unlabeled materials of comparable purity are used, and they are added in making up the stock solutions or in diluting to the concentration of the final treatment solution. In the latter instance two stock solutions of each compound, one labeled and one unlabeled, are required.

As an example, if one were using the stock solution mentioned above, having 1.105 mg per ml of labeled material, and if the treatment solution is again to contain 0.05 μc per 10 μl and to have the specific activity of not 10 but 0.5, then to prepare 1 ml of treatment solution, he takes 100 μl of his original solution, plus 100 μl of a stock solution of the unlabeled 2,4-D having 20.995 mg per ml. After evaporation of the volatile solvent, these aliquots, totaling 2,210 μg of 2,4-D, are made up to 1 ml with the proper solvent, usually 50 per cent ethyl alcohol containing 0.1 per cent Tween 20. Preparation of 1 ml of treatment solution in this case is simply a matter of convenience; smaller quantities may be prepared by using proportionately smaller aliquots.

The materials used in preparing the treatment solutions include a piece of heavy waxed paper to cover the working space; the labeled and the unlabeled stock solutions; a box of facial tissue; a set of self-filling micropipettes and a rubber single-neck syringe bulb, ¼-oz. capacity; a graduated $\frac{1}{10}$-ml pipette and a pipette control, ¼-ml capacity; culture test tubes, 10×75 mm, in a wooden test-tube support, some corks, and stopcock lubricant; a few 50-ml beakers and medicine droppers; and the surfactant stock solution if a surfactant is to be used (fig. 12). Instead of a pipette control, we have found a 4-foot length of gum rubber tubing, $\frac{3}{32}$-inch inside diameter and $\frac{1}{32}$-inch wall thickness, fitted to the $\frac{1}{10}$-ml pipette to be satisfactory for pipette control; this also prevents any danger of sucking nonvolatile solutes into one's mouth.

Procedure. Transfer the labeled stock solution into the culture test tube, using a micropipette or a graduated $\frac{1}{10}$-ml capacity pipette and pipette control. To manipulate the micropipette, a rubber single-neck syringe bulb is readily adapted by burning a pinhole through the top. The transfer of acetone solutions (2,4-D and 2,4,5-T) with the graduated pipette must be made carefully and rapidly because vapor pressure will build up in the pipette and force some of the contents out of the pipette. In the case of the acetone solutions, first transfer the necessary volumes of both the labeled and unlabeled stock solutions into the test tube in which a 50 per cent alcoholic treatment solution is to be prepared. Rapid evaporation of the acetone is effected by means of a small stream of air. After the acetone has evaporated, dissolve the 2,4-D with 95 per cent alcohol, using as much volume as possible without exceeding 50 per cent by volume of the final solution. Solution of 2,4-D in 50 per cent alcohol is very slow. The aqueous and the alcoholic stock solutions are directly diluted in the preparation of the treatment solutions. In the dilution procedure, observe the microliter volumes of all the ingredients and make the solutions up to volume by adding the solvents. Label each of the treatment solution test tubes and place them in a wooden test-tube support (fig. 12). Store the treatment solutions in the refrigerator.

Rinse the contaminated graduated pipette by running the proper solvent

through the top, using a medicine dropper. Drain the waste solution from the pipette onto a wad of facial tissue. When preparation is completed, wrap the waste material in waxed paper to be disposed of by burning.

Application of the Solutions

Translocation experiments can be conducted in the field as well as in the greenhouse; however, on rainy days or days of very low humidity and wind

Fig. 13. The trunk of a small toyon tree immediately after treatment of the inner active phloem.

there may be technical difficulties at the time the solution is applied. Otherwise, the procedure is the same in the field and in the greenhouse.

Before the solutions are applied, lanolin rings are built up around the spots to be treated. The spot may be on a horizontal leaf or a leaf held in a horizontal position by means of props and strips of masking tape, or it may be on a vertical surface such as a tree trunk. In the latter case the outer bark is carved away and the active phloem layer is exposed. The active phloem layer can often be distinguished from the outer layers by its greater translucency. One knife is used to cut away the outer layers, another for the inner; the latter must be razor-sharp. Proper exposure of the inner, active phloem, without cutting through it, is difficult but can be done. It is advisable to use small trunks 1½ to 2 inches in diameter so that the bark is relatively thin yet extensive enough to allow for several treatment spots around the trunk (fig. 13). Also, if cross sections of the trunk are taken, a series of them can be autographed on a single film. Bark treatment experiments of this sort, lasting

a day, usually do not require control samples because the treatment is localized and the movement of the chemicals is primarily longitudinal. A bark sample or a cross section will have some areas free of any trace of the labeled chemicals. In the case of a bean leaf, the midrib at the base of the blade, where treatment is to be applied, may be horizontally disposed but is often U-shaped. In all cases lanolin sticks well and confines the applied drop without penetrating or damaging the contact area.

Procedure. For applying lanolin, a hypodermic syringe, 2-ml capacity, without the needle, is a convenient container and applicator. Warm the lanolin to melting and fill several hypodermic syringes. When temperatures are above 90° F, lanolin can be stiffened by mixing approximately equal parts of lanolin and starch. This stiffer mixture cannot be pushed through the hypodermic syringe, but it can be applied with a wire loop. The drop of treatment solution is applied with a 10-μl self-filling micropipette. One drop is adequate for most small plants, but the volume relationship of the chemical to the plant body cannot be overlooked. Large plants being tested for thoroughness of distribution will require correspondingly greater amounts. Where necessary, the treated spot may be protected from any disturbance by a ring made from a 2-mm section of Tygon tubing, ¼-inch inside diameter, which can be affixed with lanolin. The ring may be covered by sticking a piece of cellophane on top with lanolin. For repeated applications the piece of cellophane is easily handled with tweezers. For single applications the ring can be built up to accommodate the drop or drops of the solution, and a piece of cellophane can be placed directly on the lanolin ring. The closure over the ring should not be airtight, for rapid absorption of the solution may thus be impeded.

The Number of Plants of an Experiment

The number of plants must not exceed the total bulk that can be lightly packed into the tray of the freeze-drying tank. A control plant should be included, however, for it often produces a slight image that must be taken into consideration in interpreting the autographs.

Treatment Time

Treatment time has ranged from zero to a month, and problems of redistribution in trees may well require sampling at monthly intervals for a year or longer.[9] At 80° or 85° F in the greenhouse, bean plants have translocated traces of 2,4-D* from the base of the primary leaf into the stem in one hour.[10] Under similar conditions, 2,4-D* applied in the middle of the leaf blade of *Zebrina* is translocating into the stem in two hours.

Though traces of herbicidal chemicals can be absorbed and translocated over the entire length of a 1-foot-tall plant in three hours, a time series is often conducted to determine the tissues or regions where accumulation occurs and the eventual pattern of distribution. Accumulation at the growing regions may continue for several days after application of the treatment solution. All of the chemicals that are phloem-translocated are subject to continued distribution. Therefore, for rapidly growing greenhouse plants a

[9] O. A. Leonard, personal communication, 1956.

[10] J. Pallas, unpublished data, 1956.

time series may include 2, 4, and 8 hours, 1, 3, and 10 days, and longer if desired. The time series is an extremely useful routine preliminary test. Only one or two well-selected plants are needed for each treatment period.

A concentration series is also a very useful preliminary test to conduct with each plant species and chemical under investigation. The treatment period can be 8 or 24 hours. This series is usually 1, 5, 25, and 125 μg of the labeled chemical per 10 μl of solution. If its radioactivity is 0.5 to 2.0 mc per mM, the usual 4-week exposure is used. If it is very much higher, the exposure time can be reduced accordingly, or the radioactivity can be reduced by dilution

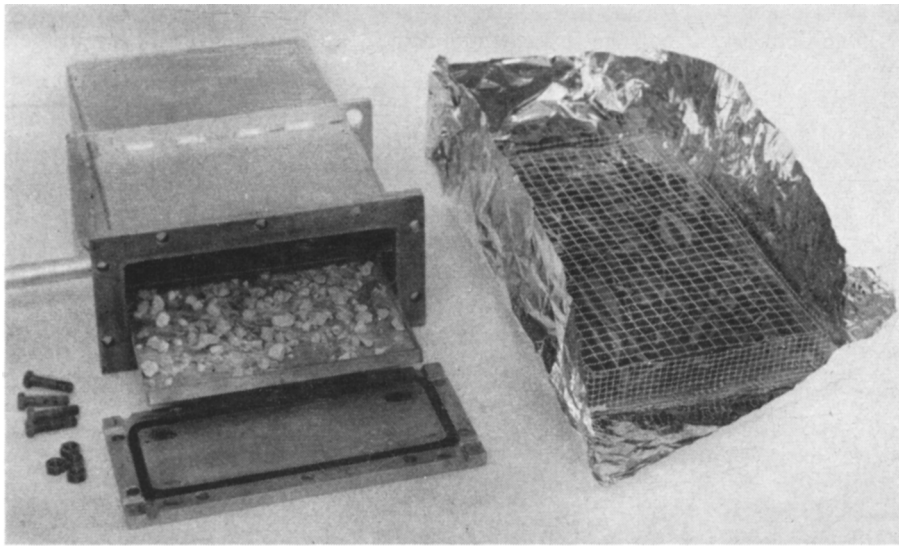

Fig. 14. The vacuum tank unit, showing the lower tray containing calcium hydride lumps and the upper tray containing plants ready to be freeze-killed. The aluminum foil wrapping around the plant tray is pulled down at the front to show construction of the tray.

with the unlabeled chemical. In all time series and in short treatments up to about four hours, it must be remembered that at temperatures of 70° F and below, the rate of absorption is markedly lower than at 80° F and above.

Freeze-Killing and Freeze-Drying

Freeze-drying in the vacuum tank progresses very differently from drying under unfrozen conditions. There is never a general, partial drying. Drying usually begins at various points at the edge or in the middle of the leaves, at the roots, and at the nodes. From the nodes drying progresses, spreading over the entire cross-sectional area into the petioles and the internodes. A partially freeze-dried plant is completely dry in some parts and not dry at all in others. Internodes and petioles are the last to dry. It is possible to take advantage of this mode of drying, and once all free liquid in the vascular tissues is removed, a partially freeze-dried plant can be taken out and air-dried; several days of drying time may be saved. If the nodal regions are dry and only the internodes and petioles remain to be dried, there is no movement of fluids from internode to internode while the plant material dries in the air.

Our present freeze-drying setup includes a custom-built vacuum tank unit (fig. 14), the freezing compartment of a domestic refrigerator without the automatic defroster, a moisture trap in the vacuum line, and a Duo Seal vacuum pump, made by Welch Manufacturing Company, which pulls a vacuum of ½ μ. To protect the vacuum pump from corrosion and loss of vacuum, the vacuum line should contain a vacuum trap for moisture and an absorption trap for acidic and alkaline vapors. For convenience, a vacuum gauge, such as the Universal 3-Scale McLeod type, should be included. The upper tray of the vacuum tank holds the plant material to be freeze-dried. The tray is made of hardware cloth.

The temperature of the freeze-drying tank is set at –7° C. If the temperature is set any higher, there is danger of partial thawing during the freeze-drying process or when the tank is taken out to be checked. If the temperature is set much lower, the drying time will be unnecessarily lengthened.

Procedure. Shortly before the end of the treatment period, wipe off the lanolin rings with facial tissue. Place the waste in a paper bag to be burned. Then cover the treated spot with masking tape; do not let the masking tape extend over the edges of the leaf. Each plant should bear proper indentification. Remove the plants from the culture jars. Blot the roots and wrap them in paper towels to prevent radioactive contamination. Put the plants in a box to be taken to the laboratory where they will be freeze-killed.

Wrap a sheet of freezer aluminum foil around the bottom and sides of the upper tray of the vacuum tank, which is to hold the plant material. The edges of the foil should extend about 3 inches above the top of the tray for stagnation of carbon dioxide (fig. 14). Put the tray on the workbench and, discarding the paper towels, place the plants in it, one by one, so that the space is uniformly occupied. Confine all parts of the plant material to the tray. If confinement is difficult, place a piece of ½-inch-mesh hardware cloth over the plants and tie or hook it to the edges of the tray. All arrangement of the plant material must be completed before freezing in order to avoid any loss by breakage later. There should be enough space between the plants to pour in pulverized dry ice for quick freeze-killing.

A 50-pound package of sliced dry ice is enough to freeze-kill the plants for one experiment. It is pulverized in a heavy wooden box, with a mallet. Pour the pulverized dry ice into the tray so as to completely surround and cover the plant material. Pour gently to prevent breakage of frozen leaves. The moment the dry ice is poured over the plants is considered the end of the treatment time, since the plants are frozen almost instantly.

Take the vacuum tank from the freezer compartment of the refrigerator and remove the lid. Take off the aluminum foil wrapping, and place the tray in the vacuum tank. Take out the lower tray and spread out about 150 gm of calcium hydride lumps per square foot. (Do not handle calcium hydride with bare hands. A wet hand can be severely burned almost instantly.) Since calcium hydride will powder upon decomposition and occupy several times the volume of the original material, leaving the unexpended lumps in the bottom, it is useless to spread out more. Sprinkle some pulverized dry ice over the calcium hydride, and place the tray in the vacuum tank. Secure the lid on the vacuum tank and return the tank to the freezer compartment of

the refrigerator. Do not jar it on the way or the plant material will shatter. Allow ample time for the dry ice in the vacuum tank to dissipate; it is useless to operate the vacuum pump during this time, and the carbon dioxide may add to the corrosion of the pump. Then hook up the vacuum line and turn on the vacuum.

If the amount of plant material is large in proportion to that of the calcium hydride lumps, the rapid accumulation of calcium hydroxide powder on the lumps will almost stop the drying process on the third day. If the plants are not dry, it will be necessary to replenish the calcium hydride. To do this, remove the vacuum tank from the refrigerator, releasing the vacuum slowly

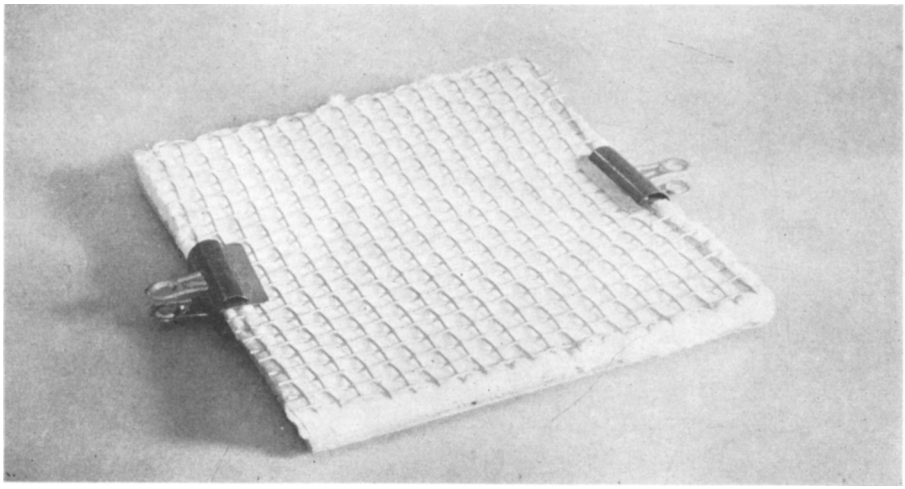

Fig. 15. Plant material prepared for rapid drying by the oven-drying method.

so that the plant material is not blown around and shattered. Be careful of wet surfaces when handling the calcium hydride lumps or the expended material—there may be enough calcium hydride left to constitute a fire hazard. Over a sheet of thick aluminum foil screen out the unexpended lumps, using ⅛-inch-mesh hardware cloth. Dispose of the waste. Return the unexpended lumps to the original tray, add more lumps and some dry ice, and return the tank to the refrigerator for continued pumping. The vacuum tank should be checked about every three days until one becomes familiar with the drying time and the behavior of calcium hydride. The oil in the vacuum pump must be changed after each month of operation.

In place of calcium hydride for trapping moisture, a low-temperature condensation, such as a large-capacity condensation flask, placed in the vacuum line and bathed in a mixture of acetone and dry ice, is equally good.

Oven Drying

Oven drying at 80° to 85° C can be used sometimes when the treatment period has been longer than a day, if the most critical evaluation of translocation and distribution is not required. Translocation, absorption, and diffusion processes in the plants will have reduced the amount of freely mobile labeled

chemical to a level of very low significance in the case of 2,4-D* (Pallas, 1957). The tendency would be the same for other chemicals. Drying time for bean plants is about an hour, for *Zebrina* an hour and a half.

Procedure. Set out a piece of plywood, $\frac{1}{4} \times 10 \times 12$ inches. Place on it a piece of cushion cut to 10×12 inches. This may be cut from various sorts of $\frac{1}{2}$-inch-thick packing material or from cotton batting. Place on the cushion a double piece of filter paper folded to 10×12 inches. Insert the plant in the fold, spreading it out in the way that it is to be mounted. Place on top a 10×12-inch piece of $\frac{1}{2}$-inch-mesh hardware cloth. Clip two opposite edges of the set with large stationery clamps (Hunt Clip No. 3); see figure 15. The pressure of the hardware cloth against the plant keeps it flat as it dries.

Open-Air Drying

Bark samples, which have not been used in short treatments, have been open-air dried rather than freeze-dried. A sample of thin bark, such as that peeled from the trunk of a manzanita tree, is held flat between two screens, with a sheet of paper placed between the cambium face of the bark sample and the screen. A thick bark is first trimmed down to about $\frac{1}{8}$-inch in thickness, and the bark sample is then removed from the tree and dried between screens.

Since the process of bark sampling itself damages the translocating tissues and there is a small amount of movement of fluids, a slight deviation from the actual condition in the intact plant is inherent in the process. For this reason, bark samples are not taken much before 24 hours of treatment time have elapsed. By this time the deviation that may occur from sampling is negligible. The results of this method have been consistent, and there has been no indication of any artifact (Leonard and Crafts, 1956).

Mounting the Dried Plant Material

The mounting procedure includes dampening the dried material to make it flexible, mounting it on paper, flattening, and redrying. A 5-gallon X-ray developer tank provides an adequate humidifying space. For mounting, white paper the size of the film and of the substance of heavy writing paper is most suitable.

Many greenhouse plants require only two or three hours to dampen. Bark samples often take several hours. The dampened plants dry out rapidly on hot days and need to be redampened two or three times before mounting is completed. On cooler days such drying is not a problem. Since mounting is slow and plant material must not be so moist that fluids will move when the pressure is applied, it is needless to dampen more than a few at a time.

Procedure. Set the tank on its side and place a shallow tray of water in the bottom. Above the water tray arrange three or four shelves of hardware cloth. Remove the tray of freeze-dried plants from the vacuum tank. Carefully separate the plants and place each one on a separate sheet of paper. Place three or four plants in the humidifier, leaving each on its sheet of paper.

When the plants are sufficiently dampened, mount them with rubber cement, leaving a 1-inch margin at the bottom of the paper for entering details of the experiment. Any excess rubber cement on the paper can be sponged off before it dries with facial tissue folded several times. Since the

labeled chemicals can easily be rubbed off any part of the plant material, all measures should be taken to prevent contamination of the rubber cement source and the mounts. Enter the identifying details at the lower right-hand corner with soft pencil so that they can be read in red light.

Always mount the treated side of the leaf against the paper, without removing the masking tape. After the specimen has been mounted, put a sheet of paper over it and roll the stems and nodes with the head of a mallet or a

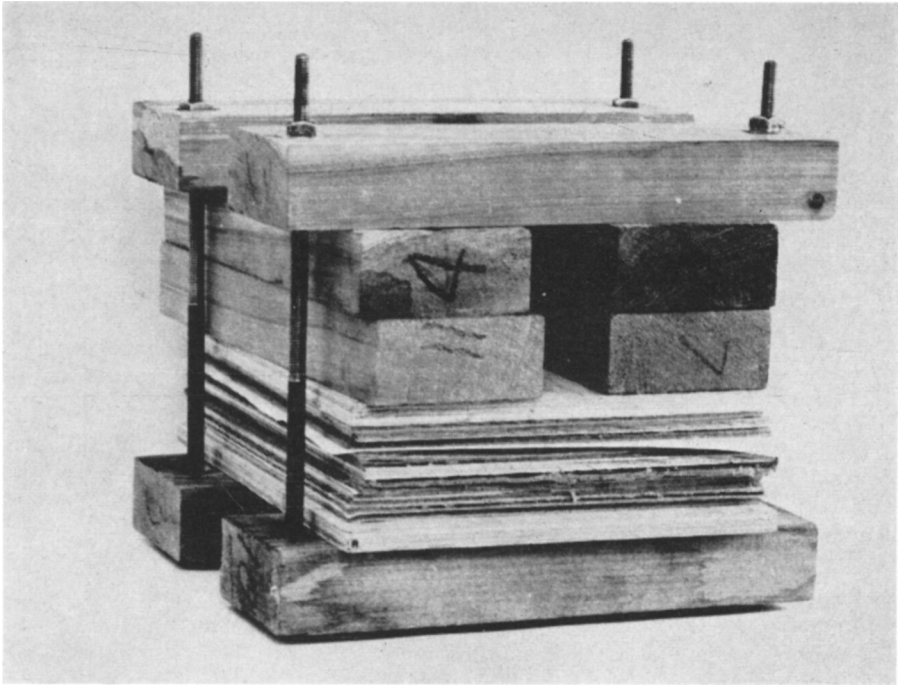

Fig. 16. The press in which plant material is flattened in preparation for autographing.

rolling pin to flatten them. Then replace the sheet of paper with a clean one. Place the mount face down on a piece of plywood cut to $10\frac{1}{4} \times 12\frac{1}{4}$ inches. Put a $\frac{1}{4}$-inch thickness of blotters on top. Now sandwich this stack of the plywood, the mount, and the blotters between two heavy pieces of board, $10\frac{1}{4} \times 12\frac{1}{4}$ inches, and clamp the entire stack tightly between four 2×4's, using four bolts (fig. 16).

Mounts are treated individually so that they can be put in the press while still somewhat flexible. If the process of clamping and unclamping is too time-consuming to repeat for each one, the mounts can be allowed to dry. Then, when mounting is finished, remoisten the entire set of mounts by clipping them on film holders and suspending them in empty photographic wash tanks containing a small amount of water and with wet paper towels hung alternately between the mounts. Cover the tanks. When the mounts are sufficiently dampened, the entire set can be stacked at one time, and clamped.

Overnight in the press is adequate. Disassemble the clamps; take the cover

papers off the mounts slowly; lay the mounts out to dry thoroughly. In the meantime examine the mounts for any sticky masses of rubber cement, which will blemish the autograph. They can be removed by rubbing them off lightly with small pieces of masking tape. Any thin, nonsticky coating of rubber cement can be left on. Air the mounts for a day to minimize pseudoautographing, which is produced not by radioactivity but by volatile emanations from the plant during exposure of the film.

Bark samples are treated a little differently. They go directly from the humidity chamber to the press. Lay out a piece of board and place a sheet of paper on it; place the bark samples on the paper, inner side down; place another sheet of paper over the bark samples, a ¼-inch thickness of blotters over the paper, than another piece of board. Lay a sheet of paper on the board, and the stack is ready for the next set of bark samples. Clamp the final stack as before.

Overnight in the press is also adequate for the bark samples. Remove the bark samples from the press; trim them for size and thickness. Without dampening them, mount them, like other plant material, with rubber cement. These are not pressed again. Enter proper identification in bold letters at the lower right-hand corner. These mounts are now ready to be autographed.

Autoradiographing

The autographing equipment for one bundle, or one set of mounts, includes several pieces of ¼-inch-thick sponge rubber cut to the size of the film; several pieces of ¼-inch plywood, 10¼ × 12¼ inches, covered with aluminum foil; two pieces of ¾-inch plywood, 10¼ × 12¼ inches, for the bottom and top boards of the stack; and two web belts.

The sponge rubber does not give off vapors reactive on the emulsion of the film, nor does it transmit carbon-14 rays at the intensities used. It is used to compensate for the differences in thickness of the plant material and to press the mount even and as close as possible against the film. The plywood provides a flat, rigid backing for the film; otherwise, black spots will appear in the film at the pressure points. Covering the plywood with aluminum foil is necessary to shield the film against vapors emanating from the wood and reacting with the emulsion.

Our standard film-exposure time is four weeks (Crafts, 1956*a*). Thin material, such as leaves, treated with 1.0 μc or more of labeled chemical will produce a satisfactory tracing of translocation in less than four weeks of film exposure. Proper adjustment of exposure time is of prime importance in the autographic procedure. With insufficient exposure time the presence of even very high radioactivity in the veins of a leaf would hardly show up. With excessive exposure time not only would the veins be black, but there would also be a dark haze around the vein (see fig. 17). This haze resembles an image that could easily be produced by a diffusion type of movement of the labeled chemical from the vein, but it is apparently due mostly to the exposure of the film to lateral rays, as is generally the case when the intensity of radiation is overly high or when the exposure period is too long. This haze is inevitable around the treated spot and in the treated leaf, but it is not of great concern because the radiation intensity and the exposure time are set primarily to

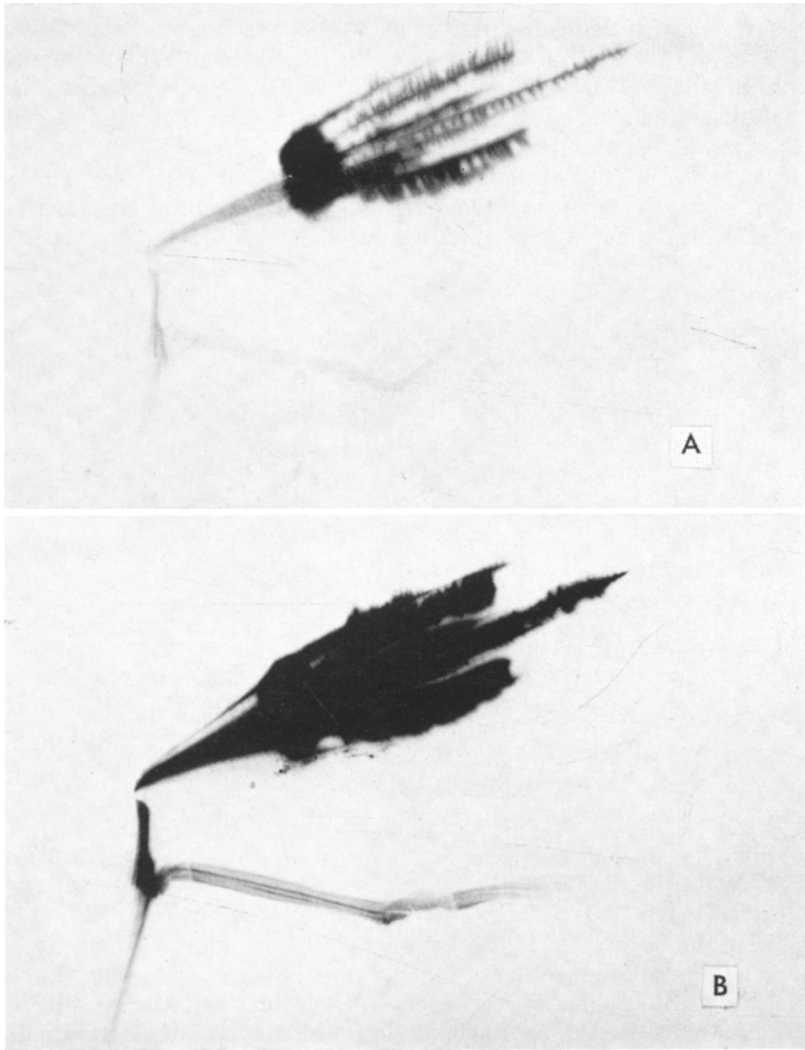

Fig. 17. A comparison of autographing exposure times of (*A*) 2½ days and (*B*) 26 days. In (*A*) note details of fine veins of the leaf. In (*B*) note the vascular bundles in the stem. Exposure time should be scaled to show the desired details in the plant material. Treatment consisted of 0.55 μc of 2,4-D-1-C^{14}, 6.03 mc per mM, one drop of alcoholic solution on the lower surface of a *Zebrina* leaf; treatment time 3½ hours.

trace translocation and distribution in the entire plant or over a large distance.

Procedure. Prepare the autographing stack in the darkroom at a distance of 10 to 15 feet from the light source (a 25-watt red light bulb). Place two web belts on the workbench; place a piece of the ¾-inch plywood across them and then an aluminum-covered board on top of it. Now the autographing stack is ready for the first folder of film and the mount.

The X-ray film we use is Kodak No-Screen X-ray film. It has emulsion on both sides, and each film comes individually enclosed in a folder. Lift up the top cover of the folder and the film by the lower edges and insert the mount between the film and the bottom cover of the folder. Lower the film onto the mount so that the flattened side of the plant material comes in direct contact with the film. Transcribe the identifying details on the mount to one corner of the film, with lead pencil, saving one-half inch of the corner for the clip of the film hanger. Bring the cover of the folder down over the film, turn over the folder with the enclosures, and place it on the autographing stack. The film side of the folder is thus backed by the board. Now place a sheet of sponge rubber on the stack to cushion the mount side of the folder. When the plant

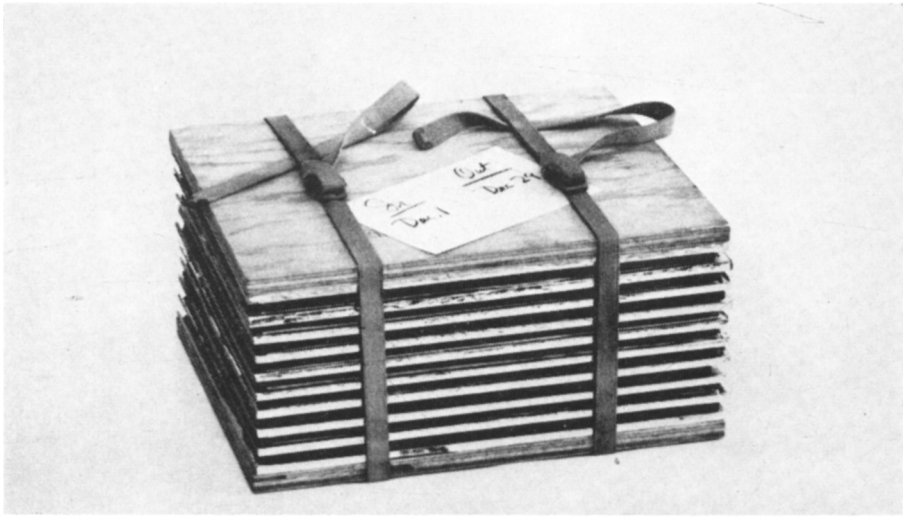

Fig. 18. Folders, each holding a film and plant mount, are sandwiched between sheets of sponge rubber and plywood. The bundle is ready for autographing exposure.

material is thin, both sides of the sponge rubber can be used to cushion the mounts, and now another folder of film and mount can be placed on the stack. This time, place the folder with its enclosures on the stack without turning it over. Then place a board on the stack to back the film side of the folder. Thus the stack is built up in the sequence of board—film—mount—rubber—mount —film—board—film—mount, etc. Complete the stack by placing the other piece of ¾-inch plywood on top (fig. 18). Tighten the belts around the stack to make a rigid bundle. Close contact between the film and the plant material is desirable, but excessive pressures, as are produced with clamps, will blacken the film at the points of high pressure (see Leonard and Crafts, 1956, fig. 3). Attach a card to the bundle and enter the "in" and "out" dates. Attach a similar card to the box. Store the bundle in a light-tight box kept in the darkroom, attaching a similar card to the box.

After the film is developed according to the standard procedure for X-ray film for normal contrast, allow the autographs to dry and then return them to their original folder in the same position as during the exposure. Insert

a sheet of lithograph paper cut to the size of the autograph between the autograph and the mount. This serves as an adequate white backing to show up the image of the autograph without using a light-box. Lettering directly on the film can be done satisfactorily with a Speedball pen (size B-6) and India

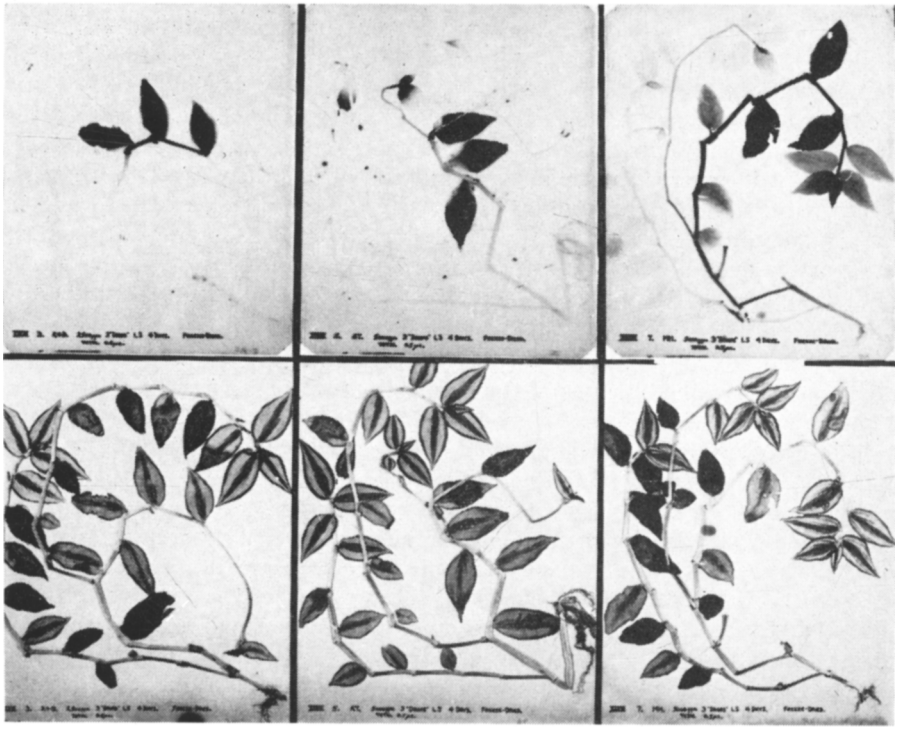

Fig. 19. Results of a comparative test on *Zebrina* plants of labeled 2,4-D (left), aminotriazole (center), and maleic hydrazide (right). Upper row, autoradiographs; lower row, mounted plants. In each case three leaves were treated and these show black in the autographs. The photograph was taken with films against a glossy white paper background. Two Eastman Vari-Beam stand-lights, Model 1, 115 volts, 500 watts, were used; these were placed to illuminate the plants and films from the sides.

ink. Enter at the bottom of the film, in abbreviated form, all of the details essential to the interpretation of the autograph (fig. 19). For example:

XVI 3. 2,4-D. 2,500 ppm. 3 drops US 4 hrs. Freeze-dried.
Total 0.50 μc.

would be the entry for Experiment No. XVI, plant No. 3, which had received three drops of 2,4-D at 2,500 ppm, applied on the upper surface of a leaf or leaves. The three drops contained a total of 0.50 μc. The treatment time was four hours, and the plant was freeze-dried.

Filing of the Autographs

The original mounted plant material and its autograph are generally kept in the same folder. The convenience of such an arrangement is somewhat offset by the necessity for careful handling. An efficient filing system for storage is

essential to prevent any unnecessary abrasion, flexing, and handling. All of the folders of one experiment are placed in one manila folder adapted to fit closely over the set of folders of the experiment.

Procedure. To adapt a manila folder for this use, first make another fold so that the V-fold is made into a U-fold of the proper width. Cut two squares about 2×2 inches from another manila folder. Fold them into U-shaped troughs of the same width as the manila folder. Spread one out and enter on the back the experiment number and other convenient identification. Glue it onto the manila folder so that one end is partly boxed at the outer edge. Glue the other piece onto the other end, close to the folds. Glu-Bird cement, made by Acorn Adhesives Co., Inc., dries rapidly and sticks very tight. The experiments can then be filed in standard-size filing cabinets. To keep the files from falling over in the drawer and to provide easy removal and replacement of any particular folder, U-shaped partitions to fit the drawers can be made by making two right-angle bends 6 inches apart in 12×18-inch sheet metal.

Reproduction of the Autoradiographs

Black and white photographs of the autoradiographs are very unsatisfactory if taken by transmitted light as through ground glass. If taken by reflected light, however, they can be sharp and detailed. Illumination should be from the sides only and with the angle of incidence at 45 degrees or less. A white background is necessary. Smooth, glossy white paper is preferred for background material. Since the image of the autoradiograph will cast a shadow on the white background, the background must be in direct contact with the autoradiograph so that there is no room for shadows. The white background material and the autoradiograph are brought into direct and uniform contact by sandwiching them between a layer of cotton batting and a plate of glass. The glass will reflect the image of the camera and other reflecting surfaces in front of the glass. The camera should, therefore, be behind a black cloth with a hole just for the lens.

ACKNOWLEDGMENTS

The methods described in this paper have been developed by a number of workers, including Charles McCarthy, Mrs. Barbara Kean, Douglas Stewart, James Pallas, Jr., and Dr. O. A. Leonard, as well as the authors.

LITERATURE CITED

BIDDULPH, S.
1956. Visual indications of S^{35} and P^{32} translocation in the phloem. Amer. Jour. Bot. **43**:143–148.

CALVIN, M., ET AL.
1949. Isotopic carbon. Chapter 3. New York, J. Wiley.

CLOR, MAHMOOD A.
1951. Studies on the translocation of 2,4-D in cotton plants. Unpublished master's thesis, University of California, Davis.

COLWELL, R. N.
1942. The use of radioactive phosphorus in translocation studies. Amer. Jour. Bot. **29**:798–807.

CRAFTS, ALDEN S.
1956*a*. Translocation of herbicides. I. The mechanism of translocation: methods of study with C^{14}-labeled 2,4-D. Hilgardia **26**(6):287–334.
1956*b*. Translocation of herbicides. II. Absorption and translocation of 2,4-D by wild morning-glory. Hilgardia **26**(6):335–365.

DAY, B. E.
1952. The absorption and translocation of 2,4-dichlorophenoxyacetic acid by bean plants. Plant Physiol. **27**:143–152.

FANG, S. C., and JOSEPH S. BUTTS
1954. Studies in plant metabolism. III. Absorption, translocation and metabolism of radioactive 2,4-D in corn and wheat plants. Plant Physiol. **29**:56–60.

HAY, J. R., and KENNETH V. THIMANN
1956. The rate of 2,4-dichlorophenoxyacetic acid in bean seedlings. I. Recovery of 2,4-dichlorophenoxyacetic acid and its breakdown in the plant. Plant Physiol. **31**:382–387.

LEONARD, O. A. and A. S. CRAFTS
1956. Translocation of herbicides. III. Uptake and distribution of radioactive 2,4-D by brush species. Hilgardia **26** (6):366–415.

OBORN, E. T., E. T. LYONS, and L. O. TIMBLIN, JR.
1954. Studies on herbicidal action on aquatic weeds using radioactive 2,4-D-1-C^{14}. Joint report, Field Crops Research Branch of Agricultural Research Service, Beltsville, Md., and Bureau of Reclamation, Denver, Colo. May, 1954.

ORGELL, W. H.
1955. The isolation of plant cuticle with pectic enzymes. Plant Physiol. **30**:78–80.

PALLAS, J., and A. S. CRAFTS
1957. Critical preparation of plant material for autoradiography. Science **125**:192–193.

WEINTRAUB, R. L., J. BROWN, M. FIELDS, and J. ROHAN
1952. Metabolism of 2,4-dichlorophenoxyacetic acid. I. $C^{14}O_2$ production by bean plants treated with labeled 2,4-dichlorophenoxyacetic acid. Plant Physiol. **27**:293–301.

WEINTRAUB, R. L., J. H. REINHART, R. A. SCHERFF, and L. C. SCHISLER
1954. Metabolism of 2,4-dichlorophenoxyacetic acid. III. Metabolism and persistence in dormant plant tissue. Plant Physiol. **29**:303–314.

YAMAGUCHI, S., and A. S. CRAFTS
1956. Penetration of 2,4-D and aminotriazole from the upper and from the lower surfaces of the leaves of *Zebrina pendula*. Western Weed Control Conference, Research Progress Report. Sacramento, California, February 1956. (Mimeo.)

YARWOOD, C. E., and LOUIS JACOBSON
1955. Accumulation of chemicals in diseased areas of leaves. Phytopathology **45**:43–48.

4*m*-12,'58(3085)BEB

The journal *Hilgardia* is published at irregular intervals, in volumes of about 600 pages. The number of issues per volume varies.

Subscriptions are not sold. The periodical is sent as published only to libraries, or to institutions in foreign countries having publications to offer in exchange.

You may obtain a single copy of any issue free, as long as the supply lasts; please request by volume and issue number from:

Agricultural Publications
Room 22, Giannini Hall
University of California
Berkeley 4, California

The limit to nonresidents of California is 10 separate issues on a single order. A list of the issues still available will be sent on request.