Absorption and Translocation of Radioactive 2,4-DI by Bean Plants as Affected by Cosolvents and Surface Agents

John W. Mitchell and Paul J. Linder

Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture, Beltsville, Maryland

Radioactive iodine (I^{131}) has been used in tracer studies with 2-iodo-3-nitrobenzoic acid, a compound having plant growth-regulating properties (*6*). Stable 2,4-dichloro-5-iodophenoxyacetic acid (2,4-DI) and various derivatives of this acid were originally synthesized by the U. S. Department of Agriculture, and these were found to have plant growth-regulating activity (*5*). The method by which bean plants absorbed and translocated these stable iodo-phenoxy compounds appears, on the basis of visible responses, to be similar to that involved when they absorbed and translocated 2,4-dichlorophenoxyacetic acid (*1*). Methods of preparing radioactive 2,4-dichloro-5-iodophenoxyacetic acid (2,4-DI^{131}) and various derivatives in a radioactive form have also been described (*2, 5*).

In the present investigation a simple method of using these radioactive iodo-phenoxy compounds in the form of salts, esters, and acid has been devised and used to determine (1) the distribution of the acid and the morpholine salt in bean plants following absorption of the compounds by the leaves, and (2) the effect of different adjuvants on the rate at which these compounds are absorbed and translocated when applied to the leaves.

An applicator was designed with which measured volumes of a solution of isotopically tagged growth regulators were applied to the leaf surfaces of plants. By turning a threaded rod, pressure was applied to a rubber tube, one end of which was attached to a graduated pipette, the other end being closed. By this means a measured volume of liquid (0.01–0.02 ml) was forced out as a droplet on the tip of the pipette. This liquid was readily transferred by touching the tip of the pipette to the leaf surface of a plant.[1]

A cellophane disk was used in plating ground samples of radioactive plant material. A brass ring was pressed against the tacky surface of cellophane tape, and ground tissue of known weight was then evenly distributed over the tacky surface within the ring to make an even layer essentially one particle in thickness. Radioactivity of the sample was determined by means of an end-window Geiger-Müller counter, the counts being compared with those for a standard sample so that the results could be expressed in weight of 2,4-DI^{131} present in the tissue.

In studying the distribution of 2,4-DI^{131} acid[2] in plants following treatment of leaves, a weighed amount of the acid was dissolved in a measured amount of Tween 20 and sufficient water was added to make a mixture that contained 0.5% of Tween 20 and 10 μg of 2,4-DI^{131} per 0.01 ml. One-hundredth ml of the 2,4-DI^{131} acid mixture was applied to 0.5 cm^2 of surface on the upper side of each primary leaf of twenty selected bean plants. The plants used were of the Black Valentine variety. They had developed primary leaves that were about three-fourths expanded, and their first trifoliolate leaves were beginning to unfold. With a remote-control applicator, the treatments were placed within a lanolin ring stamped along the midrib about 1 cm from the petiole attachment (*1, 3*). Three days later the treated leaves were removed and discarded. Remaining parts were separated, dried

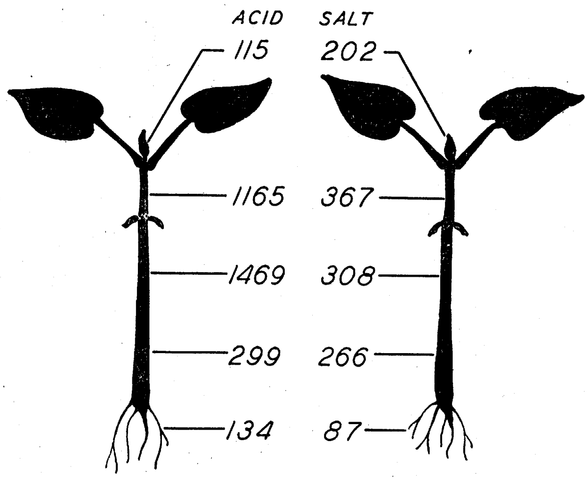

FIG. 1. Weight of 2,4-dichloro-5-iodophenoxyacetic acid (2,4-DI^{131}) and the morpholine salt of 2,4-DI^{131} in various parts of bean seedlings. Numerals represent mμg of the chemicals detected in each part 3 days after treatment of each leaf with 10,000 mμg of acid or salt.

at 80° C in a well-ventilated oven and ground to 40-mesh dimension, and the radioactivity of weighed aliquots of each sample was determined by the cellophane-disk method using a conventional end-window Geiger counter (*3*). Distribution of the morpholine salt of 2,4-DI^{131} was studied in a subsequent experiment by methods identical with those described.

An average of 3,182 mμg (total application 20,000 mμg) of 2,4-DI^{131} acid was translocated from the leaves to the main axis of plants used in the first experiment. In contrast, an average of only 1,248 mμg of the morpholine salt was moved from the leaves to the main axis of plants used in the subsequent experiment. Since these two experiments were not carried out simultaneously, the plants of each were not subjected to the same environmental conditions and this may have influenced to some extent the relative amounts of the salt and acid absorbed. The results are similar, however, to others obtained in an earlier preliminary experiment in which the rates of absorption of the acid and of the morpholine salt of 2,4-DI^{131} were compared in a single experiment. In this earlier

[1] A detailed description of all methods referred to in this paper has been published as a U. S. Department of Agriculture mimeograph by the Bureau of Plant Industry, Soils, and Agricultural Engineering, December, 1949.

[2] I^{131}, obtained from the Atomic Energy Commission, was synthesized into 2,4-DI^{131} by the Texas Research Foundation, Renner, Texas.

experiment it was found that when equimolar concentrations of the salt and acid were applied to the leaves, the bean plants translocated 23.6% more acid than salt, as was indicated by radioactivity in the stems determined after a 24-hr period of treatment.

In the present experiments, distribution of the acid and salt within the plant was similar, the greatest proportion of that absorbed being concentrated in the first internode and upper part of the hypocotyl, and the lowest in the roots (Fig. 1). Under the conditions used, an average of 17.6% of the morpholine salt, translocated to the main axis, accumulated in the terminal bud (parts above the second node); while only 3.6% of acid, translocated to the main axis, accumulated in this portion of the plants. The highest concentration of morpholine salt occurred in tissue of the terminal bud, while the highest concentration of the acid was centered in the first internode. Lowest concentrations were found in roots of plants treated with the acid as well as of those treated with the salt.

A similar experiment was made to learn whether the presence of a cosolvent, Tween 20, affected the absorption and distribution of the morpholine salt of 2,4-DI^{131}. The radioactive salt was applied as previously described except that the primary leaves of some of the plants were treated with a water solution of the salt, while others were treated with an aqueous mixture containing an equal amount of the salt and 0.5% of Tween 20.

The adjuvant increased both the concentration and the total amount of the salt found in the main axis of the plants. As a result of this increased rate of absorption and translocation due to Tween 20, the amount of the salt which moved from the leaves to the roots was increased by 71%, an effect of interest in connection with the use of 2,4-D in killing deep-rooted perennial weeds. With respect to distribution, the highest concentration of salt occurred in the terminal bud and the lowest concentration in the roots, irrespective of the presence of the adjuvant.

The effect of several cosolvents and several surface-active substances on the absorption of the radioactive morpholine salt was measured. A stock solution was prepared by dissolving the salt in water to make a concentration of 1 μg/0.1 ml. Aliquots of this solution were then used to make mixtures, each containing the same concentration of the salt and the respective adjuvants, individually. These mixtures, including the water solution of the salt without adjuvant, were then applied to the primary leaves of selected test plants as described. Two-hundredths ml of each solution was used per leaf, making a total of 4 μg per plant when calculated on an acid equivalent basis. Plants that received the nine different treatments were arranged as randomized blocks in a greenhouse with three replications of each treatment, a total of 135 plants being used. In these tests, six of the adjuvants increased significantly the amount of the salt absorbed and translocated to the stems, the most effective being Tween 20 (Table 1).

It should be emphasized that the tested adjuvants increased the rate of absorption and translocation of the salt even though the solutions were applied and confined to uniform areas of leaf surface. In this way the spreading effect of the adjuvants was limited. Effects of these and other adjuvants on the absorption of growth-regulating substances are being studied further in an attempt to learn the nature of their action.

TABLE 1

EFFECT OF COSOLVENTS AND SURFACE-ACTIVE AGENTS* ON THE ABSORPTION AND ACCUMULATION OF 2,4-DI^{131} MORPHOLINE SALT IN BEAN STEMS WHEN TREATMENTS WERE CONFINED TO A SURFACE AREA OF 0.5 CM^2 ON EACH LEAF (APPLICATION 4 μG PER PLANT; ABSORPTION PERIOD, 72 HR)

Treatment	Concentration of adjuvant (%)	2,4-DI^{131} in stems† (mμg)	Increase due to adjuvant (%)
Treated control (salt in water)		177	
Tween 20	0.5	796‡	350
Carbowax 1500	0.5	687‡	288
Emulphor-ELA	0.5	544‡	207
Repcolene	0.1	534‡	202
Igepal-300	0.1	464‡	162
Dreft	0.1	271‡	53
Nekal-NS	0.1	202	14
BRIJ-30	0.1	183	3

* Tween 20 and BRIJ-30 furnished by Atlas Powder Co., Wilmington, Del,; Emulphor, Nekal, and Igepal by General Dyestuffs Inc., N. Y.; Repcolene, by Refined Products Corp., Lyndhurst, N. J.

† Sample composed of first internode and terminal buds.

‡ Significantly greater (by 1 : 19 or better) than amount detected in control treated without use of adjuvants.

When calculated on the basis of absolute amounts in earlier experiments, radioactive 2-iodo-3-nitrobenzoic acid accumulated in greatest quantities in the terminal buds of bean plants following its application to the leaves (*4*, *6*). This is in contrast to the behavior of both the acid and the morpholine salt of 2,4-DI^{131} which, on the basis of absolute amounts, accumulated mainly in the first internodes and upper part of the hypocotyls. R. W. Holley has reported by personal communication that the distribution of radioactive 2,4-D applied to the leaves in bean plants was similar to that reported here for 2,4-DI^{131}.

The growth regulator of the benzoic acid type (2-iodo-3-nitrobenzoic acid) apparently reacted with some constituents in the relatively immature tissues of the terminal bud more readily than with those in the more mature parts of the stem. In contrast, the phenoxy compounds, especially the acid, reacted most readily with constituents of the relatively mature stem tissues.

References

1. HOLLEY, R. W., BOYLE, F. P., and HAND, D. B. Unpublished manuscript.
2. LINDER, P. J., MITCHELL, J. W., and WOOD, J. W. *Science,* 1950, **111**, 518.
3. MAHLER, H. R., SPEER, R. J., and ROBERTS, A. *Science,* 1949, **110**, 562.
4. MITCHELL, J. W. and LINDER, P. J. *Methods used in tracing radioactive growth regulating substances in plants.* Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Dep't Agr. mimeograph, 1949.
5. MITCHELL, J. W. *et al.* *Science,* 1947, **106**, 395.
6. WOLFE, W. C. *et al.* *J. org. Chem.,* 1949, **14**, 900.
7. WOOD, J. W., MITCHELL, J. W., and IRVING, G. W., JR. *Science,* 1947, **105**, 337.