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ABSORPTION AND MOBILITY OF FOLIAR APPLIED NUTRIENTS[1,2,3]

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One criterion of the effectiveness of nutritional sprays is the rate at which the foliar applied nutrients are absorbed by the leaf and translocated to other plant parts including the roots. Two distinct but not easily separated processes, absorption and transport, are involved. Initial penetration most likely involves passage through the cuticle and epidermal cells (17, 20); stomata may also play a minor role (15). Transport of foliar absorbed nutrients undoubtedly occurs via the phloem (2, 3, 4, 14). While the rate and amount of transport varies with each element, it parallels the phenomenon of nutrient redistribution as described by Biddulph (2) and Williams (19). The extent of redistribution (mobility, phloem transport) of each nutrient in the plant is an important consideration in foliar applications of fertilizer to satisfy nutrient needs of plants (20), in assaying the capacity of meristems to initiate "internal starvation" (19), and in "keying-out" nutritional deficiencies (2). Symptoms of malnutrition may arise from lack of mobility as well as lack of initial absorption.

Only in a few instances and with a limited number of the essential elements have rates of foliar absorption and transport been reported (2, 7, 14, 15, 20). As with herbicides (8, 17) isotopic labeling offers a unique approach to studies of nutrient absorption, transport (export) from leaves, and mobility. The rates of absorption, by the primary leaves of the bean, of several isotopically labeled nutrient elements, and their subsequent mobilities and partitioning within plant parts are herein presented.

MATERIALS AND METHODS

A black seeded Blue Lake bean variety (Rogers Bros., Twin Falls, Idaho, Stock No. 42335) was selected as the experimental plant because of its uniformity. Seeds were germinated in coarse No. 8 quartz sand (American Graded Sand Co., Chicago). After emergence the seedlings were transferred to aerated solution cultures described by Asen, Wittwer and Teubner (1).

The radioactive isotopes, their physical and chemical characteristics, and the pH of the treating solutions used are listed in table I. Radioactive materials, both processed and irradiated units, were obtained from Oak Ridge National Laboratory, Oak Ridge, Tennessee, Nuclear Science and Engineering Corporation, Pittsburgh, Pennsylvania, or the Abbott Laboratories, North Chicago, Illinois. Solutions of each were prepared with the appropriate carrier at concentrations non-injurious to leaf surfaces, and at pH levels (table I) previously determined favorable for rapid uptake.

TABLE I

PHYSICAL AND CHEMICAL CHARACTERISTICS OF ISOTOPES AND pH OF TREATING SOLUTIONS

ISOTOPES	CHEMICAL FORM	SPECIFIC ACTIVITY	pH OF TREATING SOLUTIONS
Na^{22}	NaCl	Carrier free	4.5
P^{32}	H_3PO_4	15173 mc/gm	3.0
S^{35}	SO_4	Carrier free	3.0
Cl^{36}	HCl	0.586 mc/gm	3.0
K^{42}	K_2CO_3	20 mc/gm	9.0
Ca^{45}	$CaCl_2$	102 mc/gm	3.0
Mn^{52-54}	$MnCl_2$	Carrier free	3.0
Fe^{55-59}	$FeCl_3$	2.60 mc/gm	2.0
Cu^{64}	$Cu(NO_3)_2$	4660 mc/gm	3.0
Zn^{65}	$ZnCl_2$	148 mc/gm	3.0
Rb^{86}	$RbCl_2$	576 mc/gm	3.0
Sr^{89}	$SrCl_2$	Carrier free	3.0
Mo^{99}	$(NH_4)_2MoO_4$	101 mc/gm	8.0
$BaLa^{140}$	$BaCl_2$	Carrier free	3.0

The isotopically labeled solutions were applied within 48 hours after the plants were transferred to the solution cultures. A small drop (0.01 ml) of the radioactive solution was placed in the center on the upper surface of one of the primary leaf blades at the stage of development illustrated in figure 1. Experimental variables known to have an effect on foliar absorption of nutrients as determined in previous tests were standardized (9, 10, 14, 15, 20). Plants were grown under the long (15 to 16 hours) days of late spring and early summer, temperatures of from 20 to 30° C were maintained and all isotopes were applied from 8 to 9 A.M.

At various intervals after treatment plants were harvested and partitioned as follows (fig 1). A circular disc (A) 1 cm in diameter, was first removed, which included the spot to which the radioactive solution was applied. The plant was then separated into (B) the remainder of the treated leaf, (C) stem plus non-treated leaf and (D) the roots.

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In this paper, the term nutrient refers to what are now known as essential as well as some non-essential elements.

The plant parts were cut into small segments placed in 50-ml beakers, dried at 70° C in a forced draft oven and the dried samples assayed directly in the beakers for radioactivity using an end-window G-M tube and standard scaler circuit. Self-absorption was found negligible for all isotopes with the exception of S^{35} and Ca^{45}.

Absorption of each nutrient and its subsequent mobility and distribution was measured by determining the percent of applied isotope recovered in non-treated plant parts. This was calculated from the total radioactivity applied to each plant and the radioactivity of the individual parts. Percent absorption at a given interval after treating was calculated as the percent of the total radioactivity recovered in all plant parts shown in figure 1 exclusive of the leaf disc (A) which included the site of application.

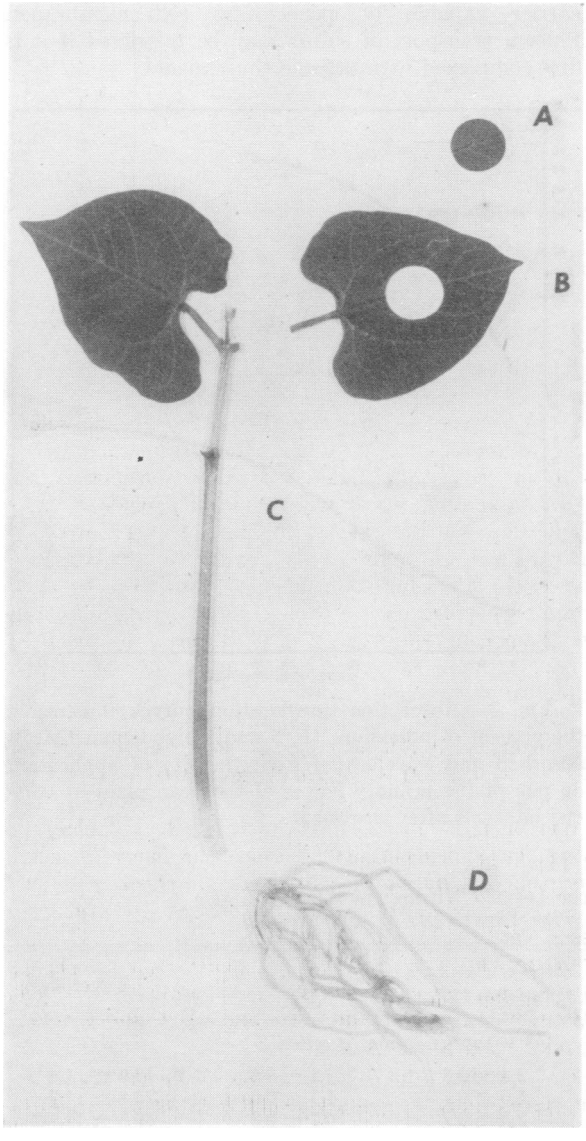

FIG. 1. Typical bean plant and parts assayed for radioactivity. A—One-cm diameter disc to which the isotope was applied, B—Remainder of treated leaf, C—Stem plus non-treated leaf, D—Roots.

It was not possible to distinguish between the absorbed isotope and that remaining on the surface of the 1 cm diameter leaf disc (fig 1-A). Therefore, the amount of each isotope absorbed at the site of application and translocated within the 1 cm leaf disc was not considered. When the entire leaf was immersed in the labeled solutions (table III), absorption of the applied nutrient was measured in terms of the percent recovered in non-treated plant parts. With both methods the absorption values were lower than the true value.

Varying the concentrations in the treating solution by as much as 10-fold for each isotope showed a highly positive correlation between the total amount of each isotope applied to the leaf and the total amount absorbed and translocated. Thus, in the tables and figures which follow, all values are expressed as percentages of the total applied.

Two techniques were used to evaluate absorption rate and relative mobility of each isotopically labeled element. The radioactivity of all plant parts was determined at intervals of 6, 24, 48, 96 and 192 hours (shorter intervals were used for the short lived isotopes, K^{42} and Mo^{99}) after application to the leaf blade. Gross autoradiography of plants 6 and 24 hours after treatment with the isotope was used to confirm the transport data obtained by direct counting and to indicate sites of accumulation.

For radioactive counting five replicates, of a single plant each, were assayed for each time interval after treating with each isotope. Autoradiograms of three plants both 6 and 24 hours subsequent to treatment, were prepared after the circular disc which included the site of application of the isotope was removed. The otherwise intact plant specimens were then dried, exposed to x-ray film and autoradiograms prepared according to methods outlined by Wittwer and Lundahl (21). The values from three separate experiments for each labeled nutrient (table I) were utilized in the tabular and graphic data which follow.

Results and Discussion

Figures 2 and 3 confirm previous reports (2, 7, 14, 15, 20) that all mineral elements are probably absorbed by leaves, but at greatly varying rates. The absorption curves (figs 2 and 3) vary from almost linear to asymptotic, with half times ranging from 6 hours or less for sodium (Na^{22}) and rubidium (Rb^{86}) up to undetermined, but much longer time intervals for iron (Fe^{55-59}) and molybdenum (Mo^{99}) (figs 2-A, 2-D and 3). The four groupings in figure 2 show that for a particular series of elements absorption and transport rates vary, but are closely parallel.

The isotopes most rapidly absorbed by the bean leaf were Na^{22}, K^{42} and Rb^{86}. Rapid uptake occurred with both methods of leaf placement (table III), and was accompanied by transport resulting in

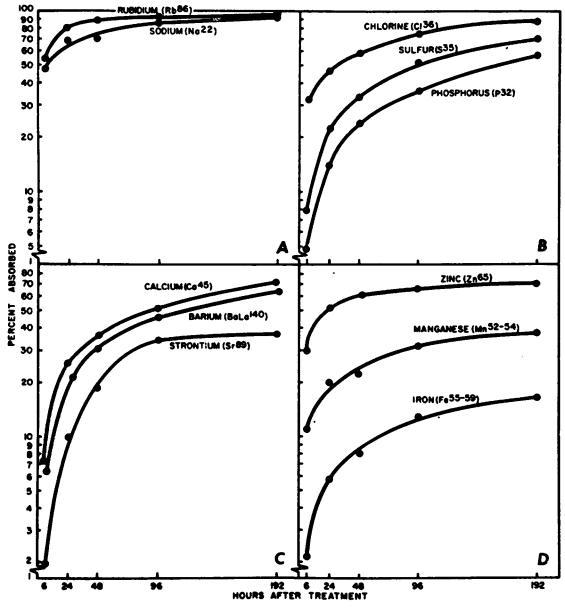

FIG. 2. Absorption–translocation curves illustrating the percent of the isotopes absorbed and translocated from the site of application on one of the primary leaves of the bean plant at 6, 24, 48, 96 and 192 hours after treatment. A—Rubidium (Rb^{86}) and sodium (Na^{22}); B—Chlorine (Cl^{36}), sulfur (S^{35}) and phosphorus (P^{32}); C—Calcium (Ca^{45}), barium ($BaLa^{140}$) and strontium (Sr^{89}); and D—Zinc (Zn^{65}), manganese (Mn^{52-54}) and iron (Fe^{55-59}).

an accumulation in the stems within 24 hours (tables II, III and fig 4), especially for Na^{22} and Rb^{86}.

Phosphorus (P^{32}) and sulfur (S^{35}) showed closely parallel absorption-transport rates (fig 2-B) with half times comparable to those already reported (7, 20). In agreement with the results of Biddulph (2) and Biddulph, Cory and Biddulph (3), transport and distribution of S^{35} compared favorably with P^{32}. More rapid leaf uptake occurred with S^{35} and Cl^{36} but their rates of export from the treated leaf were about the same as P^{32}. The usual metabolic gradient to meristems was apparent for both isotopes and is illustrated by the autoradiograms (figs 4-G and 4-H).

The distribution patterns for P^{32} and S^{35} within the bean plant are not easily distinguishable and resemble that of Zn^{65} (fig 5-B). In contrast, the anion Cl^{36} (fig 4-I) is distributed in a pattern similar to Na^{22}, K^{42} and Rb^{86} (figs 4-D, 4-E and 4-F).

That transport in the phloem may be a limiting factor in absorption and mobility of phosphorus, sulfur and chlorine is suggested by the data in tables II and III. Within 24 hours after application to the foliage high percentages (10 to 40) of the isotopes were found in the treated leaves with only minimum amounts (2 to 3 %) in the stem plus the non-treated leaf and roots. It has been suggested by Maizel, Benson and Tolbert (12), that phosphoryl choline, a constituent of plant saps, may act as a phosphorus carrier capable of penetrating cell membranes. Phloem transport of sulfur may be restricted if it is first complexed with organic constituents.

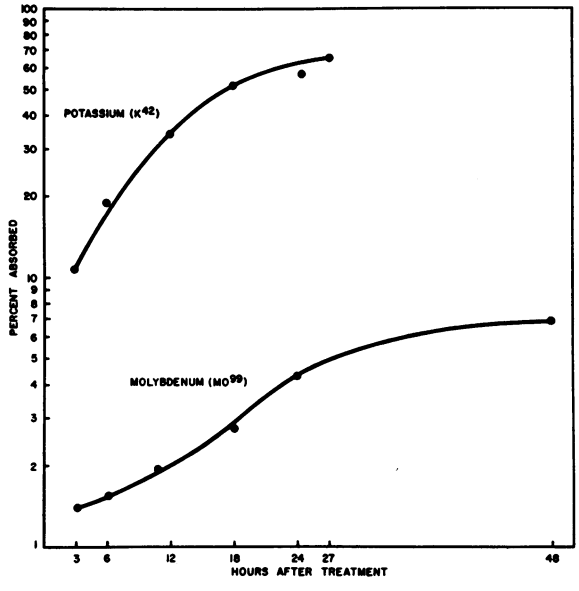

FIG. 3. Absorption–translocation curves illustrating the percent of potassium (K^{42}) and molybdenum (Mo^{99}) absorbed and translocated from the site of application on one of the primary leaves of the bean plant at various intervals after treatment.

TABLE II

ABSORPTION AND TRANSLOCATION OF FOLIAR APPLIED NUTRIENTS FROM SITE OF APPLICATION 24 HRS AFTER TREATMENT

NUTRIENT	ISOTOPE	TREATED LEAF	STEM + NON-TREATED LEAF	ROOT	TOTAL
			% total nutrient applied		
Phosphorus	P^{32}	10.5 ± 1.7 *	2.4 ± 0.3	1.1 ± 0.6	14.0 ± 1.4
Sulfur	S^{35}	20.1 ± 4.7	1.6 ± 0.7	1.1 ± 0.6	22.8 ± 4.7
Chlorine	Cl^{36}	43.6 ± 11.3	2.3 ± 0.7	0.7 ± 0.2	46.6 ± 11.7
Sodium	Na^{22}	41.4 ± 7.1	25.5 ± 8.1	1.1 ± 0.6	68.0 ± 10.3
Potassium	K^{42}	42.5 ± 2.4	9.9 ± 1.4	4.7 ± 0.7	57.1 ± 2.7
Rubidium	Rb^{86}	53.2 ± 4.8	18.4 ± 2.6	8.7 ± 4.9	80.3 ± 4.5

* Standard error.

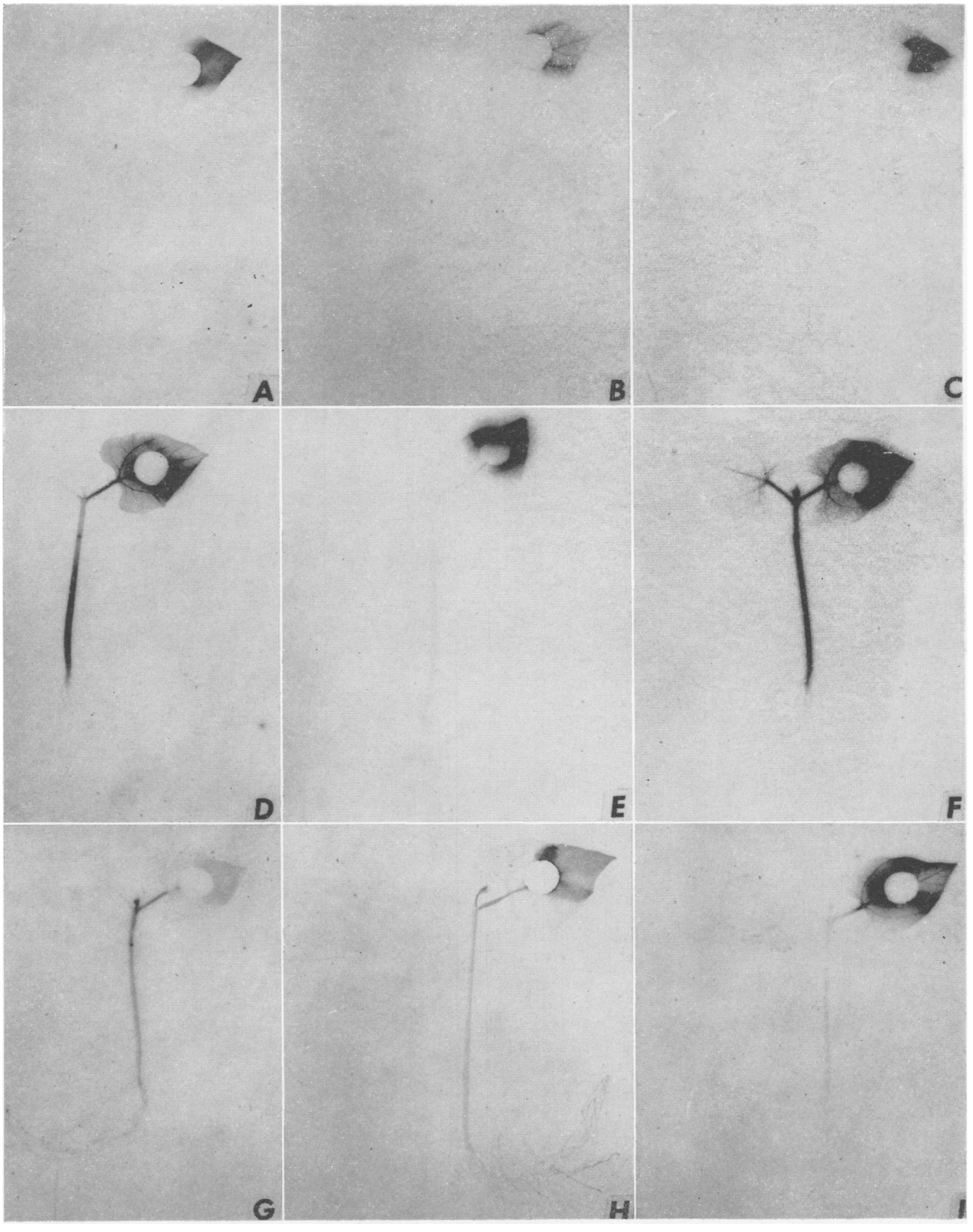

FIG. 4. Autoradiograms illustrating the distribution of Ca^{45} (A), Sr^{89} (B), $BaLa^{140}$ (C), Na^{22} (D), K^{42} (E), Rb^{86} (F), P^{32} (G), S^{35} (H), and Cl^{36} (I), 24 hours following application to one of the primary leaves of the bean plant. Since exposure times varied in the preparation of the autoradiograms, a strict quantitative comparison is not possible. (Disc removed from the leaf indicates site of isotope application.)

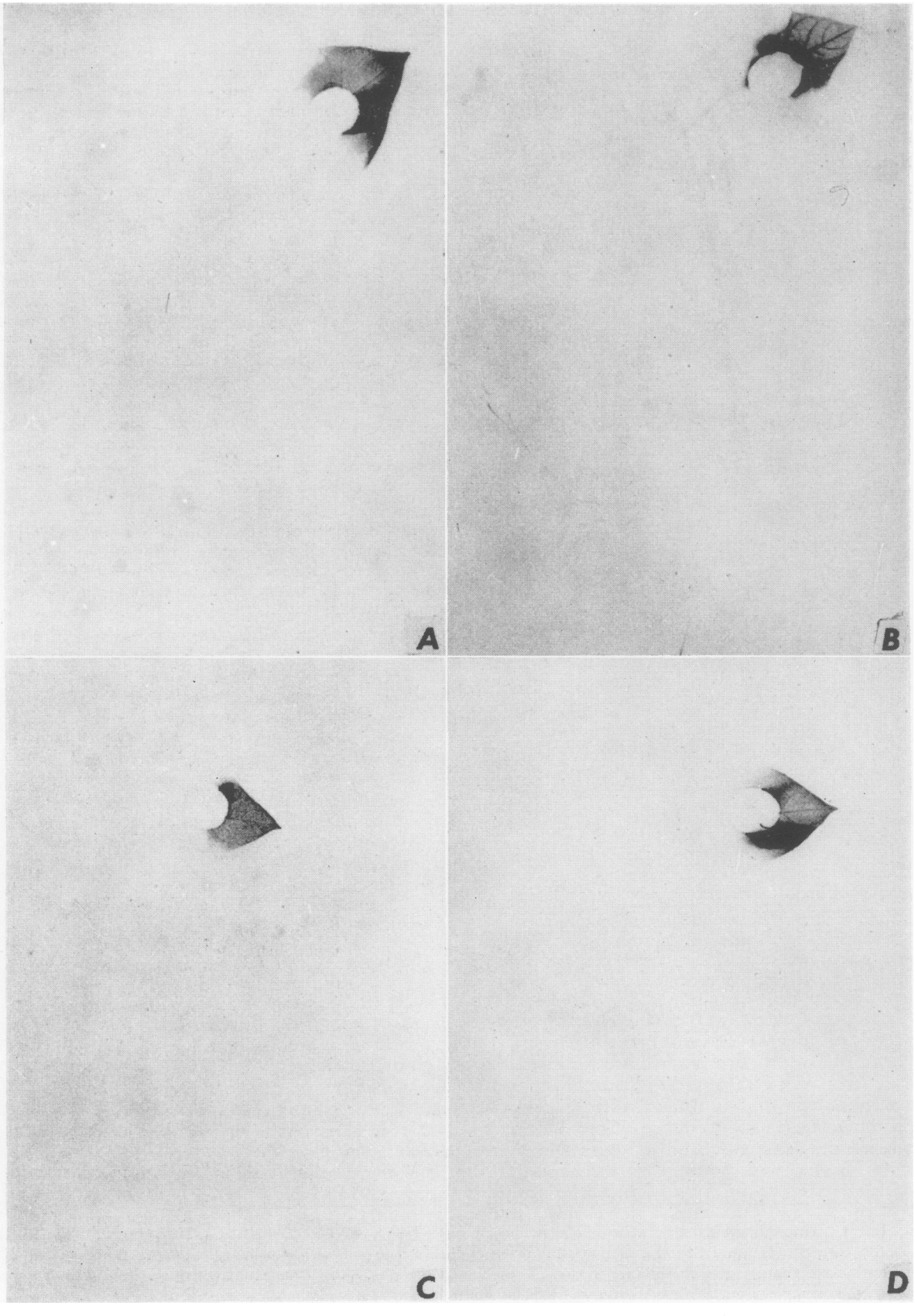
A
B
C
D

TABLE III

FOLIAR ABSORPTION * OF NUTRIENTS AS RELATED TO METHOD OF APPLICATION TO THE LEAF

NUTRIENT	ISOTOPE	LEAF DIPPED		DROPLET ON LEAF BLADE	
		STEM + NON-TREATED LEAF	ROOT	STEM + NON-TREATED LEAF	ROOT
Phosphorus	P^{32}	0.3	0.2	1.1	1.0
Sulfur	S^{35}	...	..	1.6	1.1
Chlorine	Cl^{36}	1.2	0.3	3.5	0.3
Sodium	Na^{22}	12.5	4.2	13.4	1.7
Potassium	K^{42}	11.2	1.2	10.2	0.8
Rubidium	Rb^{86}	15.2	5.8	16.1	6.5

* Expressed as % of applied nutrient recovered after 24 hrs in non-treated parts.

The absorption rates of calcium (Ca^{45}), strontium (Sr^{89}) and barium ($BaLa^{140}$) are closely parallel. Whereas transport from the site of application into adjacent leaf tissue is appreciable, export from the treated leaf is negligible. The almost complete absence of basipetal transport of Ca^{45} verifies previous reports by the authors (5, 6, 20) as well as others (2, 14). That strontium (Sr^{89}) and barium ($BaLa^{140}$) are also immobile has been determined by the absence of radioactivity in plant parts other than treated leaves, and is illustrated by the autoradiograms (figs 4-A, 4-B, 4-C). The immobility of calcium, strontium and barium is attributed to failure of phloem transport, and polar movement (20). When polarity is suspended by anesthetization with di-ethyl ether, basipetal transport (via the phloem?) occurs (5). Within the leaf, transport from the site of application occurs only toward the apex and periphery (figs 4-A, 4-B, 4-C). The possible role of transpiration in such movement has been suggested (22).

Zinc (Zn^{65}), copper (Cu^{64}), manganese (Mn^{52-54}), iron (Fe^{55-59}) and molybdenum (Mo^{99}) were intermediate in mobility between highly mobile phosphorus (P^{32}) and relatively immobile calcium (Ca^{45}), and with decreasing mobility in the approximate order listed. According to Wallihan and Heymann-Herschberg (18), zinc (Zn^{65}) was readily absorbed by and transported from the leaves of citrus. Romney and Toth (13) have reported that manganese (Mn^{54}) was absorbed through soybean leaves and translocated throughout the plants. Figures 2-D and 3 showing absorption and transport rates, and figure 5 the autoradiograms, suggest that with the exception of zinc (Zn^{65}) and possibly copper (Cu^{64}) there was little export from the treated bean leaf. Transport was largely confined to the adjacent leaf tissue and appeared to follow the transpiration stream. Appreciable basipetal transport did not appear evident up to 192 hours after treatment. Absorption values (figs 2-D and 3) for manganese (Mn^{52-54}), iron (Fe^{55-59}) and molybdenum (Mo^{99}) represent the amount of isotope recovered, almost exclusively, in the treated leaf minus the 1 cm disc (fig 1-B). Extreme variations in results precluded a listing of accurate absorption rates for Cu^{64}, however, the autoradiogram (fig 5-A) suggests that some export from the treated leaf occurred.

Several limitations are obvious in the materials and methods employed and in the results presented. 1) Processed formulations and irradiated units of the isotopes varied greatly in specific activities (table I). Thus, standardization of molar concentrations or ionic carriers of the solutions applied to the leaves was not feasible. However, this limitation was partially overcome by expressing absorption-transport values as percentages of the total isotope applied. 2) All absorption-transport rates were lower than the real values since the 1 cm leaf disc including and surrounding the site of application of each isotope was not included. Radioactivity recovered in other plant parts represented the absorbed and translocated element. 3) For absorption studies on sodium (Na^{22}), potassium (K^{42}), and rubidium (Rb^{86}), samples taken at intervals of less than 6 hours would have been desirable because of the rapid absorption and transport which occurred with these materials. 4) In the assay of plant samples for radioactivity, self-absorption of the weak beta emitters such as S^{35} and Ca^{45} by the dried plant tissue was not considered. This was determined to be of no great consequence on the nature of the results procured with the possible exception of S^{35}, since little or no Ca^{45} was exported from the treated leaf, and self-absorption by the leaf tissue itself was small. Self-absorption of S^{35} by the dried tissue undoubtedly resulted in values that were low, however, all radioactive values were relative and expressed as percentages of the total applied, and the results compared favorably with those of Biddulph, Cory and Biddulph (3) where greater precautions were taken to eliminate this variable.

It is apparent from these studies that all elements applied to leaves were absorbed and translocated but not at equal rates or in a comparable pattern. Any mass flow mechanism of transport was unlikely. The presumably free ions of sodium, potassium, rubidium and chlorine were rapidly absorbed by the bean leaf and a high percentage exported to other plant parts, especially into the stems.

While phosphorus, sulfur and possibly zinc were

FIG. 5. Autoradiograms showing the distribution of Cu^{64} (A), Zn^{65} (B), Fe^{55-59} (C), and Mn^{52-54} (D) 24 hours following application to one of the primary leaves of the bean plant. Since exposure times varied in the preparation of the autoradiograms, a strict quantitative comparison is not posible. (Disc removed from the leaf indicates site of isotope application.)

TABLE IV

CLASSIFICATION OF NUTRIENTS AS TO THEIR MOBILITY IN THE BEAN PLANT FOLLOWING FOLIAR APPLICATION *

MOBILE	PARTIALLY MOBILE	IMMOBILE
Rubidium	Zinc	Calcium
Sodium	Copper	Strontium
Potassium	Manganese	Barium
Phosphorus	Iron	.....
Chlorine	Molybdenum	.....
Sulfur	.....	.....

* Listed in order of decreasing mobility.

also highly mobile in the bean plant, either absorption or transport, or both, occurred at a slower rate than for sodium, potassium, rubidium or chlorine. Distribution of these nutrients within the plant appeared proportional to the metabolic activity of the various tissues. Calcium, strontium and barium were immobile, while copper, manganese, iron and molybdenum were readily absorbed by leaves but were only partially mobile—a high percentage of the absorbed isotope remained within the absorbing leaf, with the primary movement being toward the tip or periphery. Using as a criterion the percentage absorption and transport of the foliar applied isotopes from the site of application to the non-treated plant parts (figs 1-C and 1-D), and autoradiograms to depict distribution patterns within the plant at various intervals after treating, the nutrient elements studied are classified (table IV) in terms of mobility (percentage transport from the treated leaf into other plant parts).

SUMMARY

The absorption, transport and mobility of foliar applied radioactive isotopes of rubidium, sodium, potassium, phosphorus, chlorine, sulfur, zinc, copper, manganese, iron, molybdenum, calcium, strontium, and barium were determined with the bean as the experimental plant. Using as a criterion the percent of the foliar applied radioactive isotope recovered in non-treated plant parts, and autoradiography to portray gross distribution in the plant it was found that rubidium, sodium and potassium were the most readily absorbed and most highly mobile. Calcium, strontium and barium while absorbed by the leaf were not exported from the leaf and were considered immobile. Phosphorus, chlorine, sulfur, zinc, copper, manganese, iron, and molybdenum were intermediate with decreasing mobility in the order given.

Since the preparation of this manuscript, preliminary observations with magnesium (Mg^{28}) showed that it was immobile and its distribution following foliar application was the same as Ca^{45}, Sr^{89} or $BaLa^{140}$.

The assistance of Dr. C. C. Chen, in conducting some of the experiments is gratefully acknowledged.

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THE HILL REACTION OF RED KIDNEY BEAN CHLOROPLASTS[1,2,3]

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Red kidney beans produce large, uniform primary leaves in a short amount of time. Theoretically these should be excellent material for studying problems of leaf growth, etc. One aspect of the physiology of the leaf of interest to us was the ability of isolated chloroplasts to carry on parts of photosynthesis in vitro. We noticed that the Hill reaction activity (3, 4) of such chloroplasts was considerably lower than that of spinach chloroplasts regularly in use in this laboratory. The following report concerns our attempts to improve the Hill activity of chloroplasts from red kidney bean leaves by manipulating several variables of the leaf grinding procedure.

In the course of this study, evidence has been obtained which suggests that there are two pathways for dye reduction in the Hill reaction.

MATERIALS AND METHODS

Leaves of red kidney beans, black valentine beans, peas, oat, barley, cabbage, tomato, some ferns, sunflowers, cocklebur and vanilla were obtained from plants grown in the greenhouse. Spinach and parsley were obtained from the local supermarket. Seven to fifteen grams of leaf tissue were ground in 50 ml of buffer. When leaves were ground in the Omnimixer, it was run at 35 % of line voltage for one minute. The homogenates were strained through cheesecloth and glass wool.

The basal medium for the grinding buffer consisted of: 0.30 M sucrose, 0.01 M KCl, and 0.05 M phosphate. The pH of the phosphate was varied as indicated in the text; and other additions to the medium were made at times.

All chloroplasts were centrifuged from the original homogenate at 1000 × g for 10 minutes, resuspended in 40 ml of buffer, and washed once at 1000 × g. No matter what the grinding medium, all chloroplasts were washed and resuspended in a medium containing 0.30 M sucrose, 0.01 M KCl, and 0.05 M phosphate pH 7.3.

For Hill reaction measurements, 0.072 micromoles of 2, 3, 6-trichloroindophenol dye (Eastman Kodak), 150 micromoles of TRIS (tris-(hydroxymethyl) aminomethene) buffer at pH 7.2, and an appropriate amount of chloroplast suspension in a total volume of 3.0 ml were incubated for 45 seconds in the light in a water bath at room temperature in front of a 100 Watt light bulb. The optical density at 620 millimicrons was determined before and after exposure to the light. Light intensity was considerably over saturation. The amount of chloroplasts was varied from 0.001 to 0.010 mg chlorophyll per ml of reaction mixture, in order to give a change of 0.090 to 0.120 in optical density in the 45-second period. The unit of activity from this measurement is defined as change in optical density at 620 mμ per minute. Final activities are all shown as units per mg chlorophyll. All determinations were done in duplicate or triplicate. Chlorophyll was determined by the method of Arnon (1).

RESULTS

The initial observation was that chloroplasts from spinach were regularly giving Hill reaction rates of 25 to 40 units per mg chlorophyll, while those from red kidney bean leaves showed rates of 3 to 9 units per mg chlorophyll. On the hypothesis that conditions obtaining in the grinding process were causing chloroplast inactivation, various "protective" compounds were added to the grinding medium. The first addition found effective in raising the activity of kidney bean chloroplasts was Versene (ethylenediamine tetraacetic acid). In a typical experiment, activity of chloroplasts from a medium without Versene was 4.2 per mg chlorophyll; when Versene was used at 0.01 M in homogenizing, activity of the resulting chloroplasts was 14.2 units per mg chlorophyll. Versene (0.001 M) had no effect.

The pH of the grinding medium is of considerable

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