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THE ABSORPTION AND TRANSLOCATION OF SULFUR IN RED KIDNEY BEAN[1,2,3]

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The principal objective of this study is to determine the characteristics of translocation and the ultimate partition of sulfur between the various parts of Red Kidney bean plants. The methods employed are those of geographic tracing by means of radioactive sulfur. Data are presented on the following points: 1) the effect of nutrient sulfur content on dry matter yield and on sulfur concentration within organs; 2) the effect of pH on sulfur absorption; 3) the exchange of sulfur between various organs; 4) the rate of downward translocation of sulfur in stems.

The justification for such a study lies in the fact that only a few papers bearing on the translocation of sulfur have appeared. Rippel (13) states that in various deciduous trees there is no migration of sulfur from regions of storage to new tissues as growth begins in the spring. He classes sulfur with calcium, as a "stabile elemente." Marsh (9) also states that sulfur resembles calcium in that it is not mobilized to any great extent. Little was withdrawn from leaves of the apple prior to leaf fall. Wood and Barrien (22) found that during sulfur starvation protein sulfur decreased and SO_4 sulfur increased in grass leaves held in darkness. Sulfate was not translocated to stems and roots as were the amino acids. Wood (19) concludes that in fully matured plants sulfate is relatively immobile in leaves.

In the work of Thomas et al (16), using radioactive sulfur, there is presented definite evidence that sulfur is conducted from one region to another as it is needed for growth, being conducted as the sulfate ion. They have also shown (17) that sulfate absorbed through leaves of alfalfa plants can be translocated to the crowns and then into untreated stems.

The data presented herein add specifically to the information on the translocation and partition of sulfur between the various plant parts during the early phases of growth.

METHODS

Red Kidney bean (commercial stock) served as experimental material. The plants were grown in a Hoagland type nutrient solution as follows: $Ca(NO_3)_2$, 0.0025 M; KNO_3, 0.0025 M; KH_2PO_4, 0.0005 M; $MgSO_4$ or $MgCl_2$ to give both 0.0010 M Mg and varying amounts of sulfate sulfur, and micronutrients according to Arnon (1). Growth conditions were as follows: temp 23° ± 1° C; R.H. 60 % ± 2.5 %; light, artificial at 1000 to 1200 fc (2 daylight plus 10 soft white fluorescent tubes); aeration through sintered glass; and hydrogen ion concentrations maintained at approximately pH 6. No significant variation in absorption, translocation and partition of sulfur was observed over the pH range of 4 to 7. The least pH drift occurred at 6.

The total sulfur was determined for roots, stems including the hypocotyl and petioles, primary leaves, and trifoliate leaves (both less petioles). The composited similar parts of six plants, all grown in the same tank, constituted the samples. Each experiment, except the one represented in figure 1 D was replicated at least once. Duplicate determinations were made when sample size permitted.

In order to render the sulfur of seed origin distinguishable from sulfur of nutrient solution origin after absorption by the plant, S^{35} was added to the nutrient sulfur. This will be referred to as labeeld sulfur, or indicated by S*. Prior to use the number of disintegrations per minute per 15 mgs of BaS^*O_4 from the nutrient solution was determined. This value is designated by "a." At the termination of the growth period, during which time the labeled sulfur was being absorbed by the plants and diluted with seed sulfur already present, 15 mgs of the BaS^*O_4

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[2] The radioisotopes were acquired from U. S. Atomic Energy Commission, Oak Ridge, Tennessee.
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derived from each plant sample was analyzed for S^{35}. This value in disintegrations per minute per 15 mgs BaS^*O_4 was corrected for decay during the experiment and the value designated by "b." Then since "a" represents the original S^{35} per unit of total sulfur and "b" the final S^{35} per unit of total sulfur, the percentage composition of nutrient sulfur in each plant part is $b/a \times 100$. The total labeled sulfur and the total sulfur in each part was determined from the yield of $BaSO_4$ from each part. Oxidation of the samples was accomplished in an oxygen bomb.

Results

The Effect of Nutrient Sulfur Content and of pH on Dry Matter Yield and Sulfur Concentration: The plants grown with no nutrient sulfur, i.e., only seed sulfur, showed severe signs of sulfur deficiency, the most pronounced of which were a marked reduction in growth of trifoliate leaves with yellowing and necrotic spotting. The roots remained quite normal in appearance. A comparison of the dry matter yield of plants grown at several nutrient sulfur levels is made in figure 1 A. Sulfur, it can be seen, was not limiting at nutrient concentrations of 0.125 millimolar, or above, under the conditions of these experiments, when one liter of nutrient solution per plant was renewed each four days. At concentrations above this value absorption was essentially independent of concentration. The uniformity in the dry matter yield of each part between the nutrient sulfur concentrations of 0.125 and 1.0 millimolar indicates that 0.03 millimoles of sulfur per plant per day is adequate during the initial 12 days of growth.

There was observed a lack of uniformity in the growth response of the primary leaves which was considered to have more to do with the degree of injury suffered during the shedding of the seed coat than with the nutrient treatments. The extent of injury could not always be ascertained at the time the seedlings were selected for further use.

The amount of sulfur contained in each plant part at harvest time is shown in figure 1 B. There was a slight tendency for the sulfur content of the roots to increase with increasing amounts of nutrient sulfur so that on a concentration basis, i.e., mg S/gm dry matter (fig 1 C), it seems justifiable to conclude that a slightly ascending relationship existed. This could be due to retention of nutrient solution on the root surfaces. Roots and trifoliate leaves were higher in sulfur concentrations than stems and primary leaves. However, the roots did not adsorb sulfur as phosphorus and iron are sometimes adsorbed (Rediske and and Biddulph, 12).

There was very little variation in dry matter yield or in sulfur content of the plants as a whole, or of their constituent parts, when grown at pH values ranging from 4 to 7. Two replications of six plants per unit served as the basis for this statement. These plants were grown in the greenhouse, which was not subjected to as close a temperature, light, or humidity control as was the case for the plants represented in figure 1.

The percentage of the total tissue sulfur which was derived from each of the two sources, i.e., the seed and the nutrient solution, is shown for each plant part in figure 1 D.

The incoming sulfur of nutrient origin, which was marked with S^{35}, becomes diluted with sulfur of seed origin which had been deposited before the nutrient sulfur was made available. The time chosen for beginning the administration of the nutrient sulfur corresponded to the stage of development at which the hypocotyl had straightened, the primary leaves were well expanded and the root system fairly well formed. From this time onward growth was more rapid in the root, stem, and especially the trifoliate leaves. At the time of harvest the sulfur of nutrient origin in the various parts was approximately as follows: primary leaves 50 %, stems 66 %, roots 75 % and trifoliate leaves 82 %. Sulfur of seed origin, of course, made up the remainder (fig 1 D).

At the lowest nutrient sulfur levels the roots retained relatively more seed sulfur than at the higher levels. It is also true that the roots of those plants grown on seed sulfur only were heavier than those grown with additions of nutrient sulfur (fig 1 A). To make doubly sure of this point this part of the experiment was repeated with similar results being obtained. The trifoliate leaves of these plants were very limited in size and number and manifest severe sulfur deficiency symptoms. This is all indicative of the ability of the root to acquire seed sulfur early and retain it from the developing trifoliate leaves even to their death from sulfur deficiency. A corresponding amount of organic seed reserves was also acquired and retained to facilitate the root extension characteristic of this metabolic condition. It is necessary to assume some mechanism operating within the tissue systems which ensures the development of the root system, even at the expense of the aerial parts, when sulfur is a limiting factor.

From these results it is apparent that sulfur of seed and nutrient solution origin will not become uniformly distributed throughout the plant. The rate of turnover of sulfur compounds within tissues is too low, as is shown by the fact that after 12 days with the marked sulfur available to the root the primary leaves have fallen to only 50 % seed sulfur while the newly developed trifoliate leaves have risen to only 18 % seed sulfur. A time sequence study would be required to fully establish a turnover rate.

The extent of movement of labeled sulfur, acquired at an earlier period, into current growth made during a time when no labeled sulfur was available, can be seen by comparing the autoradiographs A and B of figure 2. Figure 2 A shows the distribution of S^{35} in the plant after the roots had been immersed in a nutrient solution containing the radioisotope for four days (from the fourth to the eighth day after the hypocotyl became erect). Figure 2 B shows a plant treated similarly but allowed four additional days of

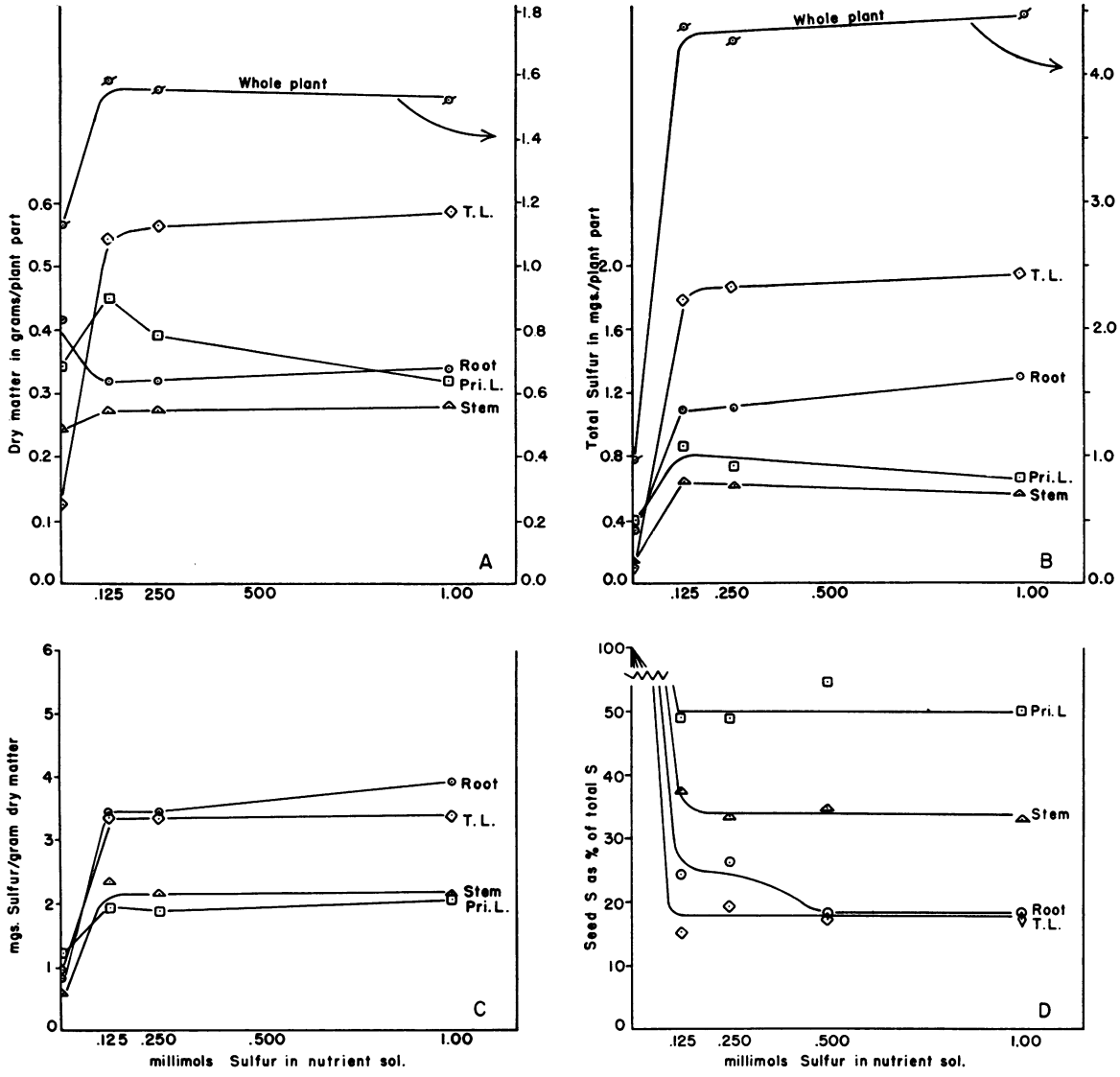

FIG. 1. A. The dry matter yield of separate plant parts, shown on the left ordinate, and of the whole plant, shown on the right ordinate, as affected by the varying nutrient sulfur concentrations shown on the abscissa.

B. The total sulfur content of separate plant parts, shown on the left ordinate, and of the whole plant, shown on the right ordinate, as affected by the varying nutrient sulfur concentrations shown on the abscissa.

C. The sulfur concentration (mg S/gm dry matter) of separate plant parts as affected by the varying nutrient sulfur concentrations shown on the abscissa.

D. The percentage of sulfur of seed origin, and conversely the percentage of sulfur of nutrient solution origin, in separate plant parts as affected by the nutrient sulfur concentrations shown on the abscissa.

growth in a nutrient solution containing no radioactive sulfur. A comparison of A and B (fig 2) shows that labeled sulfur from the root and older leaves moves into the young leaves which expand during the period when no labeled sulfur is available to the root. Since the bean root does not adsorb large amounts of sulfur it is evident that the S^{35} found in the youngest leaves must have been translocated from other parts within the plant.

The nutrient solutions employed for the whole period were of the Hoagland type mentioned earlier; so no abnormal nutritional conditions existed during any part of the experimental period. The plants were also grown under the controlled conditions listed earlier. One gets the impression from these autoradiographs that a part of the nutrient sulfur, after its absorption, remains mobile and circulates rather freely within the plant. Of the mobile fraction being moved upward by the xylem a portion will be trapped in organic synthesis, particularly in young

leaves and stem tips, while a significant portion of that normally received by mature leaves may be free to circulate. In this manner the younger leaves and the buds may be continuously supplied with sulfur, whereas under conditions of scant supply to the roots the older tissues may become deficient. This is in keeping with our observations that the older trifoliate leaves are the first to show sulfur deficiency under limited sulfur nutrition. Sulfur, in the bean, behaves like phosphorus (2) in the sequence of development of visual deficiency symptoms on leaves of different ages and positions. This is similar to the sequence in tomato (11), but contrary to that for soybean (4), black mustard (6), tea (15) and tobacco (10). Sunflower showed no gradation (5).

THE DOWNWARD TRANSLOCATION OF SULFUR IN THE STEM: A more direct method for determining the mobility of sulfur within the bean plant was used to supplement the work above. This method made possible the determination of the rate of downward movement of sulfur in the stem, and furnished as well some information on the distribution pattern of the mobile sulfur in the stem.

Twelve-day-old plants, grown as above, were used. The S^{35} was introduced into the leaf either by the leaf flap method as described earlier (Biddulph, 2) or by spraying the material directly onto the surface of the leaf. The S^{35} was carrier free and was adjusted to a pH of 4 prior to use. It assayed approximately 1 mc/ml and 50 microliters of solution were used per treatment. The treatments were begun at 2 P.M. and the migration period terminated 30 or 60 minutes later when the roots and the tops above the point of insertion of the first trifoliate leaf (the treated leaf) were simultaneously cut off. Each of the included stems was then divided into one-inch sections for individual S^{35} assays using a wet digestion procedure. In two of the trials the solution to be injected contained sucrose at a concentration of 2.5 %. This was to determine the possible effect of an enhanced sugar supply in the leaf on accelerating the translocation of sulfur. There was no appreciable influence manifest when sucrose was applied by this method. Higher sugar concentrations were tried but they invariably lowered the amount of the radioisotope which entered the leaf, and caused wilting in the vicinity of the leaf flap. When the petiole of the leaf was steamed just prior to the S^{35} application no movement of S^{35} occurred from the leaf. The results of the experimental work for this section are shown in table I.

During the thirty minute migration period the S^{35} had moved from the leaf into the root, the crown of which was 8 inches below the pulvinus of the treated leaflet. In two of the four cases reported on in table I this amounted to a rate of movement well in excess of 40 cm/hr. The lowermost section showed a relatively high activity, indicating that the front of the marked S^{35} had entered the root sometime earlier.

Figure 2 C is an autoradiograph of a bean plant treated as above (S^{35} supplied to a leaflet) except that a 24-hour migration period was allowed. It is evident that the S^{35} has moved throughout the whole plant. As a result of a rather extensive experiment it was found that such nutritional factors as high or low sulfur levels, i.e., 0.0010 and 0.00001 M, high and low calcium levels, i.e., 0.0050 and 0.0005 M, and the pH values of 4 and 7, in all possible combinations did not significantly alter the rate, amount or pattern

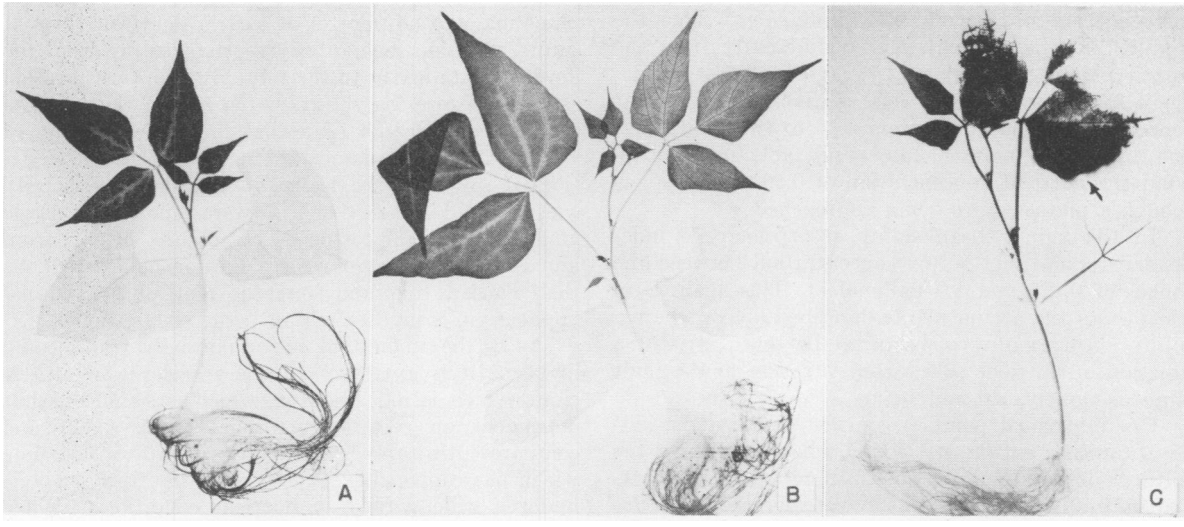

FIG. 2. A. S^{35} autoradiograph of a bean plant receiving 8.2 μc S^{35}/liter of nutrient solution (at pH 6) during the four days prior to harvest. Exposure period 90 days, to Eastman no-screen x-ray film.

B. S^{35} autoradiograph of a bean plant treated as in A but allowed four additional days of growth while no tracer was present in the nutrient medium. Exposure 90 days, to Eastman no-screen x-ray film.

C. S^{35} autoradiograph of a bean plant, the lowermost trifoliate leaf of which had been treated with 28.1 μc of S^{35}/50 μl of solution at a pH of 4. The treated leaflet is indicated by an arrow. A migration period of 24 hours was allowed and the exposure time was 60 days, to Eastman no-screen x-ray film.

TABLE I

THREE EXPERIMENTS TO SHOW THE DISTRIBUTION OF S^{35} IN THE BEAN STEM BELOW THE NODE OF THE TREATED LEAF

STEM SECTION	EXPT 1 LEAF INJECTION			EXPT 2 LEAF INJECTION		EXPT 3 SPRAY
	S^{35}	S^{35} + SUCROSE	PETIOLE STEAMED, S^{35}	S^{35}	S^{35} + SUCROSE	S^{35}
1	30,650	34,750	0	9,150	6,430	210,000
2	12,640	16,900	0	2,378	1,240	146,400
3	6,780	9,800	0	862	513	100,000
4	4,520	5,910	0	484	389	55,800
5	1,917	2,000	0	92	trace	44,500

The concentration of the S^{35} used for treatment varied between experiments but not within them. All solutions were at pH 6. Sucrose, when used, was 2.5 %. Solutions were applied to the lowest trifoliate leaf. Sprayed areas were 1 inch in diameter and on the lower surface. Migration periods: Experiments 1 and 2—30 min, experiment 3—60 min. Data are in counts per min (cpm) per one-inch section, except for the #5 sections which varied in length from 1 to 1.5 inches. Section 1 is nearest the treated leaf. The data are for single plants.

of S^{35} movement. By contrast the movement of iron can be profoundly influenced by phosphorus nutrition levels, when introduced by the same method (Rediske and Biddulph, 12).

DISCUSSION

Under the growth conditions imposed upon the plants herein studied, the dry matter yield failed to increase as the plants were subjected to nutrient sulfur concentrations beyond 0.125 millimolar. Thus the Red Kidney bean behaves very differently from the cotton plants reported on by Ergle and Eaton (8). They found increasing yields as the nutrient sulfur concentration was raised from zero to two millimolar, but the rate of increase was considerably reduced bepond the nutrient concentration of 0.50 millimolar as though a plateau were being approached.

In the kidney bean sulfur absorption was independent of nutrient sulfur concentration between the ranges of 0.125 and 1.0 millimolar. This again is in contrast to the cotton plants described above wherein sulfur absorption was shown to be quite strictly a function of nutrient sulfur concentration to the limit studied, namely, 2.0 millimolar.

One additional point of contrast between the kidney bean and cotton was found when comparing the sulfur concentration of tissues. In cotton the percentage of sulfur in leaves and stalks increases with increasing nutrient sulfur concentration to an extent which led Ergle and Eaton (8) to remark that "the tendency for the cotton plant to accumulate sulfate in its leaf cells (but not in stem cells) seems quite remarkable." In the bean the concentration of sulfur did not increase in any tissue, with the possible exception of the roots, in nutrient sulfur concentrations above 0.125 millimolar. This strict proportionality between sulfur and dry matter yield seems worthy of special mention.

A very interesting relationship among the various parts of the plant is shown in the competition among them for the sulfur contained in the seed. The first export is downward from the cotyledons through the hypocotyl to the developing root system, then upward to the primary leaves. The further development of the epicotyl is somewhat delayed, and the root will release a portion of its sulfur of seed origin to it only when sulfur from the root environment becomes available. The aerial parts, particularly the trifoliate leaves, gain an amount corresponding to the loss by the root. The consumption of the seed sulfur by root and primary leaves results in the upper part of the epicotyl being particularly low in this material as extensive tissue elaboration begins; so sulfur deficiency symptoms develop early in these upper parts. The seed sulfur within the root cells is held tenaciously and little is re-exported until such time as sulfur becomes available by absorption from the root environment. This incoming sulfur presumably allows a limited amount of turnover of sulfur compounds of seed origin which are retained by the root under conditions of sulfur deficiency. Eaton's data (4, 5, 6) led him to conclude that there was little evidence of protein breakdown and reutilization of sulfur under conditions of sulfur starvation in soybean, sunflower and black mustard. Apparently the protein bound sulfur is comparatively stable and not subject to extensive turnover. The conditions under which breakdown of sulfur containing proteins occur have been investigated by Wood and Barrien (20, 21, 22).

The normal activities of a rapidly growing bean plant under conditions of at least a moderate level of sulfur nutrition include an export of sulfur from the lower mature leaves to the root. As the transpiration stream delivers the sulfur to the leaves, there occurs a continuous though somewhat limited circulation of sulfur within the plant. In this way the concentration of sulfur in the leaves is maintained relatively constant and excesses, such as were reported by Ergle and Eaton (8) for cotton do not occur. Export from immature leaves is extremely low or nonexistent, as that which is acquired from the root, or by external application, is used locally in tissue elaborations.

As to the amount of sulfur normally translocated in beans it is greater than the translocation of calcium, which is nil, and iron, which is significant but dependent on certain nutritional factors (12) and compares favorably with phosphorus translocation, which is appreciable (2). Sulfur is freely mobile under a wide variety of nutrient conditions. When aplied to a bean leaf, either by injection or by spray application, it can be detected in all parts of the plant within thirty minutes. The amount translocated is adequate to supply at least a significant portion of the needs of the various tissues. In short, it would appear that sulfur could be at least as effectively utilized from foliar applications as is phosphorus (7, 14).

This means that foliar application could adequately supplement soil absorbed sulfur, especially where soil sulfur is limiting.

The rate of downward translocation of sulfur, shown to take place *in the phloem* (3), compares favorably with our values for P^{32}, C^{14} labeled sucrose, Fe^{55} and THO (tritiated water), each of which appears to be subject to some variation. However, this variation in rate is much less than the variation in the amounts of the different elements being translocated. The concentration of the mobile fraction in the stem is logarithmic with respect to distance traveled and is the same as would be expected if movement were by a diffusion process. The same distribution pattern has been found for mobile sulfur, phosrphorus, sucrose, iron, zinc and water, but the probable significance of these data cannot be discussed from the sulfur data alone. The resemblance of the distribution pattern to that which would be expected of a diffusion process is not, in itself, sufficient evidence to rule out any of the other theories of movement.

Summary

The data show 1) that there is a rather constant relationship between dry matter yield and sulfur content of each plant part throughout the nutrient range of 0.125 and 1.0 millimolar sulfur, 2) that trifoliate leaves and roots are richer in sulfur than stems and primary leaves, 3) that pH has little effect on sulfur uptake, 4) that a portion of the total sulfur within the plant remains mobile and moves freely from one organ to another, 5) that a portion of the seed sulfur originally translocated to the roots is released for upward translocation only when sulfur enters the plant from the root environment, and 6) that the rate of downward movement of sulfur in the phloem of the stem is similar to that for phosphorus and sucrose and exceeds 40 cm/hr.

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