Evidence for Translocation of Iron in Plants [1, 2]

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Iron is generally considered to be a very immobile element in growing plants. Localized absorption has been observed at a spot where a Fe solution sprayed on the foliage may cause a small area to become green. The fact that the surrounding chlorotic area remained chlorotic after spraying with Fe has led investigators to assume the Fe was immobile within the plant. Furthermore, workers in Kansas and Texas (6, 11) have reported that maximum yields of sorghum were obtained with 3 foliar sprays of ferrous sulfate solution. This also tends to indicate that Fe is not translocated, therefore new leaves as they become exposed must be sprayed for correction of Fe chlorosis.

Bukovac and Wittwer (2) found that about 10 % of the Fe applied to a spot on a bean leaf was translocated but this was only within the treated leaf.

Doney et al. (5) using Fe^{59} found that 25 % of the Fe applied to a leaf was translocated to the apex leaves of bean plants.

The work herein reported was designed to establish whether foliar applied Fe was absorbed and translocated, the possible pathway, and the extent of the translocation. Information on these points has important implications insofar as correcting Fe chlorosis by foliar sprays is concerned.

Materials and Methods

Sorghum plants (*Sorghum vulgare,* L. var. RS 610) were grown in soil known to be Fe deficient for this particular species. Applications of N as $(NH_4)_2SO_4$ and P and K as KH_2PO_4 were sufficient for normal growth. When the plants were in the fourth or fifth leaf stage of growth, Fe^{59} was applied in solution to a portion of a leaf. The Fe solution was 10 mM $FeCl_2$, pH 4.0, with a specific activity of about 100 μc per ml. The leaf area to be treated was enclosed by applying a ring of lanolin so that the solution would be absorbed from the enclosed area. The rates of application were 10 μl, 20 μl, and 50 μl per leaf.

Four days after treatment with Fe^{59}, the treated area of the leaf was discarded; the roots were freed of the soil by running tap water. An untreated chlorotic Fe deficient plant as well as an untreated green plant were included as controls. The plants were frozen with pulverized dry ice and freeze-dried and autoradiographed essentially according to methods described by Yamaguchi and Crafts (12). This drying method is known not to permit any internal movement of substances during the drying time.

The autoradiographs of the trial runs of sorghum showed considerable pseudoautograph, a smudging of the film in the areas of the buds and young enlarging leaves, by some volatile labile substance emanating from these organs during the film exposure time of 5 days. Therefore, the freeze-dried and mounted plants of later experiments were oven-treated for an hour at 60° and freed of the volatile substances and autographed.

Treatments of cotton (*Gossypium hirsutum,* L. var. Acala) plants were made in 2 ways. First, Fe^{59} was applied to the nutrient solution culture in which the cotton plant was growing, and secondly Fe^{59} in solution was applied to the basal region of a cotyledonary leaf blade. Treatment was for 5 days.

Bean seeds (*Phaseolus vulgaris,* L. var. Red Kidney) were germinated in perlite and grown in nutrient solution (8). To each set of 20 plants 31 μmoles of $FeSO_4$ were added 2 days before plants were transferred to a $CaCl_2$-KCl solution. After 2 hours in this latter solution pairs of plants were transferred to a $CaCl_2$-KCl solution to which had been added 40 μmoles of $FeCl_3$ containing adequate Fe^{59} for counting and autoradiographing. They remained in this latter solution for 48 hours, after which they were transferred to iron free Johnson solution (7) and allowed to grow for 26 more days.

Leaf blade activity assays were made by cutting a disc of tissue on the twentieth and twenty-sixth days after the last transfer to Johnson solution. Leaf samples from 2 folioles were counted for determining the activity variations between them. The leaf tissue was counted immediately after removal from the plants. The counting values of the twenty-sixth days sampling were corrected for decay in relation to those of the twentieth day. The distribution of iron in the plant was expressed in cpm.

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Results and Discussion

The freeze-dried and mounted plants (lower half) together with the corresponding autoradiographs (upper half) are shown in figure 1.

Sorghum. The picture in the upper left of figure 1 shows the distribution of Fe^{59} in sorghum plants following the application to a small area of a leaf. The section of the leaf to which Fe was applied was removed; this shows as a break in the leaf. Fe applied to the leaf was absorbed and translocated to other parts of the plant. A considerable portion of the Fe^{59} was translocated toward the tip in the leaf to which it was applied as shown by the image density of the fourth leaf. However, very significant amounts of Fe^{59} were transported particularly to the actively growing regions, the newly forming leaves and root tips. The leaves immediately above the treated ones show medium labelling in the mid and basal regions. These regions were enlarging at the time of treatment. Fe^{59} was carried by the assimilate stream from the treated mature leaf to all actively growing leaves and root tips. The mature regions traversed by the tracer in this translocation did not become as heavily labelled as these terminal regions of the assimilate stream. The accumulation in the bud and at the root tips would indicate transport by phloem. The higher dosage permitted proportionate or greater than proportionate accumulation in the growing bud leaves and also resulted in a little greening of the young developing leaf

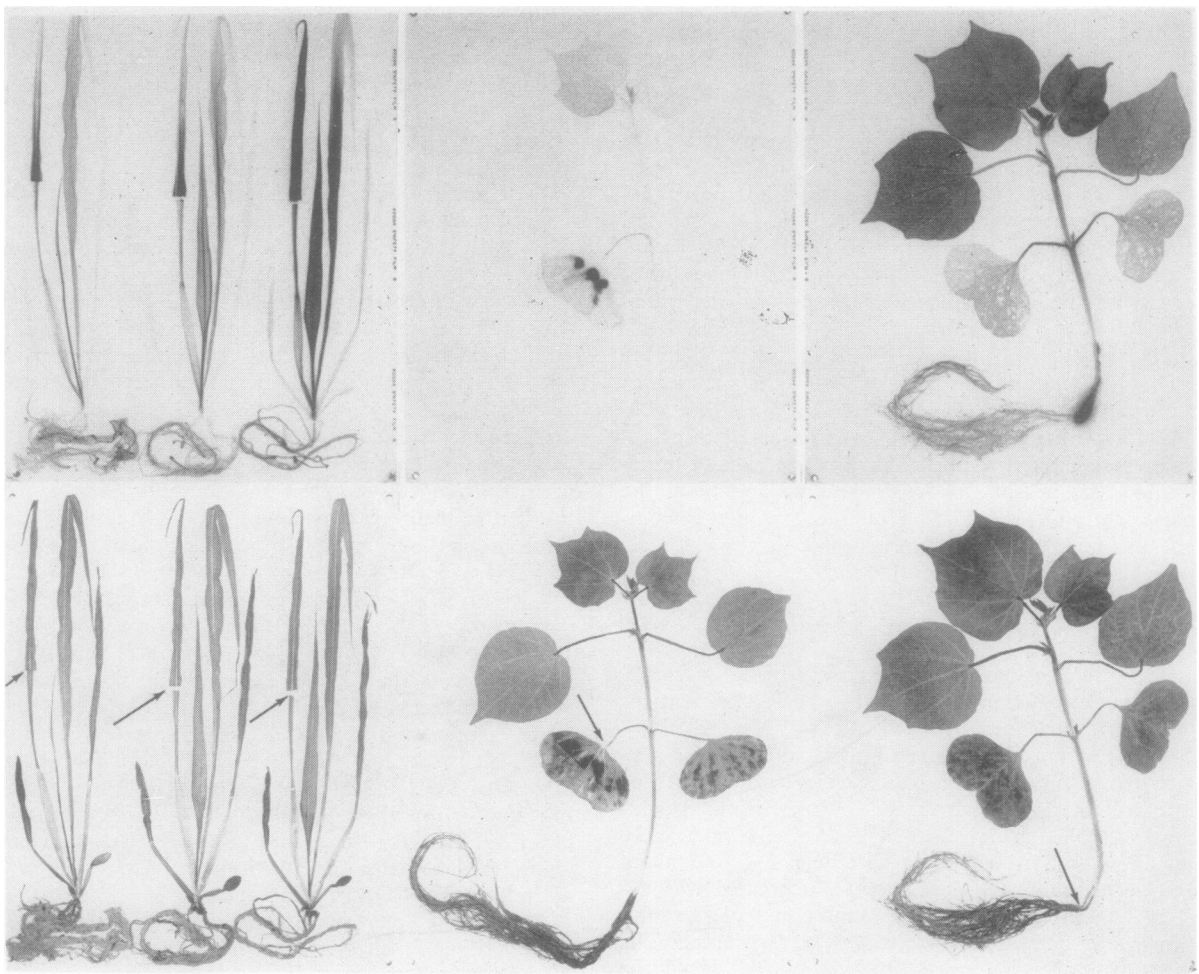

FIG. 1. Autoradiographs of Fe^{59} translocation and distribution in sorghum and cotton. Upper row, the autoradiographs of the plants shown in the lower row. Left, sorghum leaf treatments in order of 10 (1 μc), 20 (2 μc) and 50 (5 μc) μl of Fe^{59} solution. The arrows point to position whence treated regions 1 cm long, were removed. Middle, 10 (1.0 μc) μl of Fe^{59} solution (pH 4) applied in 3 droplets over the 3 major veins of 1 cotyledon; film exposure time for the treated leaf blade was 1 hour and the lighter distribution, such as that in the veins basal to the treatment, does not show; arrow points to the cut whereby the leaf blade was excluded during the film exposure, except for the last hour. If exposed 5 days like the rest of the plant, the entire leaf would be black. Right, 10 (1 μc) μl of Fe^{59} solution added to the culture solution; the arrow points to the cut whereby the roots were excluded during film exposure, except for the last hour.

which accumulated the greatest amount of activity (fig 1) upper left frame, right plant.

The maintenance of image intensity in the bud and at root tips has resulted from continued movement of the tracer; whether by translocation from the treated spot or by redistribution from maturing regions which were assimilate sinks at the time of treatment.

The light labeling of all the mature leaves, including the tip region of the leaf above the treated leaf, is from movement in the transpiration stream, directly or as a result of leakage from the phloem. Some leakage into the xylem, together with a strong phloem movement has been characteristic of very mobile substances, such as maleic hydrazide (4), and phosphate and sulfate (1).

Cotton. The distribution pattern of Fe^{59} in a cotton plant following application to a cotyledonary leaf is shown in the autoradiograph, upper center of figure 1. Here again while much of the applied Fe^{59} remained in the treated leaf, some has been transported to newly forming leaves.

The intermediate leaves received only very slight amounts of Fe^{59}. This is in great contrast to sorghum in which phloem transport was marked. In the treated leaf of cotton there has been some movement toward the leaf margin.

The primary distribution of Fe^{59} by phloem has been slight. The small bud leaf shows a little higher concentration of activity than the next 2 older leaves. This may be due to some continued phloem translocation, redistribution, or dilution of the original accumulation due to leaf expansion.

The distribution pattern of Fe^{59} in a cotton plant following application to the roots is shown in the upper right of figure 1. In this autograph the roots had 1-hour film exposure rather than 5 days. There was no evidence of a coating of Fe deposits on the roots. Whether or not root uptake and distribution of Fe in the shoots would be different if the pH were greater than that used (pH 3.5) in the present experiments can only be surmised. There were no symptoms of injury; growth of the plants was vigorous throughout the treatment period.

The distribution of activity in the mature green leaves (cotyledons and the next 2 leaves) was not uniform. The veins and especially the petiole of the cotyledons show accumulation. The 3 top leaves, being much darker than the lower ones, must have received Fe^{59} by phloem, and also by xylem because Fe^{59} is xylem-mobile, as revealed by the image of the cotyledons and the next leaf above which were fully developed at the time of treatment. The distribution of activity in the upper leaves is more uniform.

The maintenance of a little higher concentration of activity in the bud indicates some continued transport, whether by redistribution from the mature and maturing leaves or directly from the root. At any rate, the limited evidence in cotton is for high apoplastic mobility and low, but very definite symplastic mobility.

Red Kidney Bean. The concentrations of Fe^{59} in the various leaves of the bean plant are shown in table I. The counts are higher in the newest tissue, hence there is a tendency for Fe to be translocated to the actively growing regions. It was observed that the older leaves were normal green in color, while the newest growth was usually quite chlorotic.

The 2 branches, whose Fe^{59} concentrations are included in table I, grew during the time following Fe application. Thus their Fe supply must have been due to redistribution within the plant. Again the newer leaves on these branches have the higher concentrations.

At the second sampling 26 days after Fe treatment, young leaves developing from buds in the axils of at least 2 leaves, contained radioactive Fe. This Fe also must have been derived from other parts of the bean plant.

Autoradiographs prepared from one of these plants treated in the same manner, showed a similar distribution pattern of Fe^{59}.

Contrary to what has been reported frequently, Fe appears as a mobile element. Mature leaves translocate Fe to new growing points and these compete for the available Fe.

It is not possible from the results of these studies to determine which mode of transport is the more important. The significant fact is that Fe either applied to the root in nutrient solution or applied to the foliage may be transported fairly rapidly to other parts of the plant, particularly to the actively growing regions. This may explain why Fe applied to a spot on a leaf does not cause the whole leaf to become green. The absorbed Fe is rapidly transported through the conducting tissues, presumably in the phloem, to the ter-

Table I. *Iron-59 Activity Found in Leaf Disc Samples from Various Leaves of a Red Kidney Bean Plant 20 Days after Treatment*

The data are expressed in counts per minute and are averages for 2 discs, only 1 sample from 1 leaflet.

	Leaf number						
	Primary leaf	1st	2nd	3rd	4th	5th	6th
On main axis	435	560	485	335	510	1035	1450
On branch from 1st leaf node		435	775	845			
On branch from 2nd leaf node		610	760				

minal growing tissues. Chlorotic leaves do not recover readily, particularly if they have reached a more or less mature condition.

The distribution of iron via the xylem and other apoplastic regions is probably well enough understood, but the distribution of iron via the phloem is relatively new information. By phloem there is the possibility of continual redistribution within the plant. Direct evidence of Fe^{59} transport in the phloem is lacking in this communication, however, the interpretation of the distribution pattern is based on direct evidences accumulated from transport in phloem and xylem separated from each other over a short distance, Stout and Hoagland (10), Yamaguchi (13); from sugar concentration analyses above and below a phloem girdle on the main stem of cotton, Mason and Maskell (9); and from phloem exudate analyses, Crafts and Lorenz (3), Zimmerman (14).

Summary

Translocation of Fe was studied using Fe^{59} in sorghum, cotton and beans. Applications were made to the foliage and in nutrient culture solution.

From the autoradiographs and counting it was apparent that Fe was reasonably mobile under the conditions of the experiments. Fe was translocated by phloem and xylem and moved particularly to the actively growing young developing tissues.

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