Redistribution of Potassium, Calcium, Magnesium, and Manganese in the Plant

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Abstract

The extent of redistribution in apple was described by calculating the fruit/leaf ratio of the cation content. It was found that the redistribution diminishes in the sequence K > Mg > Ca ≈ Mn. These results are more or less in agreement with those found elsewhere.

Investigations were made to see whether it was possible to account for the differences in redistribution by the phloem by means of the solubility of these cations in the sieve tube sap. As model plants *Yucca flaccida* and *Ricinus communis* were used, plants from which it was possible to obtain phloem sap in a rather pure state. It was found that the addition of potassium and magnesium as a chloride in the usual investigated range of concentrations did not give precipitation. With calcium and manganese, however, a precipitate soon occurred. Manganese was demonstrated to be less soluble than calcium. For Ricinus the maximum amount of calcium and manganese the sieve tube sap could contain before precipitation set in was higher than for Yucca sap.

The results confirm the possibility that the redistribution of the different cations in the plant can be related *inter alia* to their solubility in the sieve tube sap.

It was also found that the calcium in the phloem sap is present in ionic condition. Thus the normal laws of solubility should be applicable.

Introduction

The capacity for redistribution ("mobility") of several inorganic elements in the plant has been found to show large differences. The mobility, as demonstrated in this process of secondary transport, can be determined by measuring the translocation of a radioactive element from leaf to leaf or from leaf to fruit. In general we may assume that transport in the sieve tube system is mainly responsible for the observed movements. This is indicated by the fact that it is mainly in opposite direction to translocation in the xylem.

From such determinations it has become clear that calcium and manganese can be qualified as highly immobile elements (Price 1970, Crafts and Crisp 1971), while potassium is very mobile. Magnesium is sometimes considered to be mobile (Price 1970), while in other cases it is regarded as immobile (Mengel 1965). Bukovac and Wittwer (1957) consider manganese as a moderately mobile element. Ishizuki (1971) utilized the extent of the export from the dying leaves of rice as a standard for mobility. In a certain period an export of 53%, 40% and 6% was observed for magnesium, potassium and calcium, respectively.

To explain the differences in capability of being redistributed through the sieve tube system one should have a better knowledge of the process of vein-loading, as it determines the entry of matter into the phloem. In this respect it would be important to know the route and manner in which an ion is transported through the parenchyma from xylem to phloem. But even without this knowledge we can make some hypotheses as to why the extent of redistribution differs for different ions (Fischer 1967).

The possibility exists that vein-loading is governed by the metabolically activated rate of entry of the ions. The rate determining step can either be at the moment of passage into the sieve tubes or into the symplast, embracing both mesophyll and phloem. In the last case no special barrier is assumed between the parenchymatic protoplasm and the sieve tubes. In the passage of a membrane a discriminating influence could be exerted by means of specific carrier molecules. Fischer accepts an analogy with the uptake in the root and assumes the presence of one carrier for calcium, strontium, barium and lithium and another carrier for potassium, rubidium and caesium. Within each group there could be mutual competition between the cations, while the rate of transport would be of the same order of magnitude.

It could also be conceived that the mobility is influenced by the extent of fixation of an element. Strong fixation to cellular structures of the parenchymatic tissue, such as cell walls, or in the vacuoles, would render large proportions immobile.

Another possibility to explain the differences in mobility would be a different solubility of the ions in the sieve tube sap. An easily soluble element in this sap could, in the process of sap formation, be accepted in higher concentration, and thus be transported through the phloem in relatively larger amounts.

We have tested the latter possibility for a few elements. The results of the investigations on solubility have been compared with the differences in extent of transport of the ions from leaf to fruit in apple. As it is important to know, for judging the results, if the elements exist in ionic or chelated state, this supposition was checked for one of the elements, *viz.* calcium. For this purpose the calcium in the phloem sap was tagged by addition of ^{45}Ca either *in vitro* or *in vivo*. By means of chromatography and electrophoresis of the sap it could be determined whether this element occurs in ionic state or in chelated condition.

Materials and Methods

1. *Plant material*

a. Apple

The extent of redistribution in apples was determined by means of the fruit/leaf ratio of their ionic contents. The samples were obtained from a pot experiment with Cox's Orange Pippin on M IX, which included four lime treatments on river clay soil. Besides this, some data on leaf mineral content in the autumn were derived from samples from two field experiments with Cox's Orange Pippin. Leaf samples were taken of the third and fourth leaf from the base of the new shoots which had been formed that year. As far as possible all fruit samples consisted of equally large apples.

b. Yucca

The phloem exudate was obtained from Dr. P. M. L. Tammes (Centre for Plant Physiological Research, Wageningen). After decapitation of the inflorescence stalk of *Yucca flaccida* Haw., he collected the sap in the usual manner (Tammes and Van Die 1964). Immediately afterwards it was frozen and stored and dispatched later on. After thawing the sample it was thoroughly mixed and centrifuged at about 16,700 *g*. The supernatant was then divided into smaller portions and frozen. For the investigation a small portion was thawed, heated for ten minutes at 90°C to prevent callose formation later on, and again centrifuged. Thawing and centrifugation appeared to give a loss of about 0.3 mg/l calcium. A loss of 0.6 mg/l calcium before the actual determination may thus be assumed.

c. Ricinus

Ricinus communis L. was cultivated on nutrient solution according to Steiner (1961) with equivalent ratios for K/Ca of 60/30 and 20/70. When the plants were about three months old, an incision was made in the bark according to Milburn's method (1971) and the sap drained off via a glass capillary. The daily obtained quantity of exudate, which was probably rather pure sieve tube sap, was frozen and stored. In this case also the exudate obtained during a rather long period was mixed for use in the experiments. The sap was further treated in the same way as described for Yucca exudate. It was noticed that, during centrifugation, less precipitate was formed than in the Yucca sap.

To obtain Ricinus sap labeled with ^{45}Ca, the plants were cultivated in the usual manner on a nutrient solution with a K/Ca ratio in equivalents of 60/30. After two months about 500 μCi ^{45}Ca was added to the solutions. In the period of 2 to 7 days after addition, phloem sap was collected during a few days.

2. *Chemical analysis*

The magnesium, calcium and manganese determination were made by means of an atomic absorption spectrophotometer and potassium was determined flame spectrophotometrically. The calcium determination in the sieve tube sap was found to be rather difficult, because of the low calcium content and the presence of high potassium and phosphate concentrations. Based on the recovery of added calcium, the following method appeared to be best. The phloem sap was diluted with water in a ratio 1:1 after addition of $SrCl_2$ to obtain a concentration of 1.6% and of HCl to a concentration of 0.1 *M* in the final solution to be analysed. The extinction in the atomic absorption spectrophotometer was compared with standards to which an equivalent amount of $SrCl_2$ and HCl had been added.

3. *Determination of the maximum solubility of the cations and of the quantity of precipitate*

After putting 1 ml of the phloem exudate into a glass microcuvette, stepwise additions of 0.01 ml of chloride solution in water of the element to be examined was added. The ultimate addition generally amounted to 1.5×10^{-3} mmol/ml sap. After each addition it was examined by turbidimeter whether precipitation existed and if so, its quantity. In order to precisely determine the point of incipient precipitation and to avoid too large an increase in volume, solutions of different concentrations were used. More dilute solutions were used near the point of precipitation. In calculations the increase in volume by the additions is neglected.

After an addition, the solution was always shaken; the extinction was measured after four minutes waiting time. A second extinction measurement was performed after a second shaking and three minutes waiting. Phloem sap to which equally large additions of water had been added was used as a blank. The extinction measurements were per-

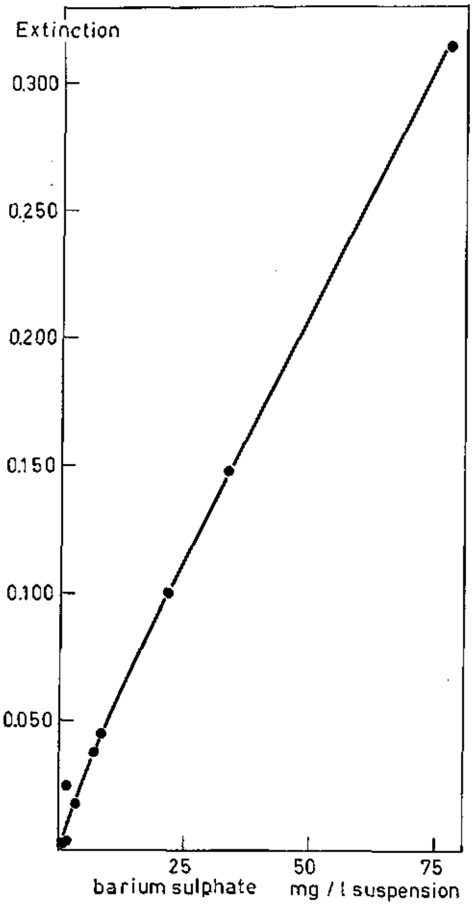

Figure 1. *Relation between the concentration of a $BaSO_4$-emulsion in 15% saccharose solution in water and the extinction measured at 400 nm.*

formed on a Zeiss spectrophotometer PMQ-II at 400 nm. It had become apparent earlier that the addition of salt hardly changed the colour of the sap samples.

Figure 1 shows that, for a barium sulphate suspension in a 15% saccharose solution, an almost linear relation exists between the suspended quantity and extinction. Accordingly a similar relation for our experiments was assumed to exist.

4. *Research into the mode of occurrence of calcium in the phloem sap*

a. Preliminary research on the addition of ^{45}Ca to Yucca sap *in vitro*

To 1 ml Yucca sap 0.01 ml of a 0.0025 *M* calcium chloride solution, containing 5.4 μCi ^{45}Ca, was added. After that the mixture remained in the refrigerator for one night. Subsequently a sample was examined by thin-layer chromatography in two directions with solvents according to Kluge *et al.* (1969) and also by paper electrophoresis at pH 8.2. The chromatograms were examined by means of their autoradiograms, while the electropherograms were analysed by a scanner.

b. Electrophoresis of the radioactive phloem sap of a Ricinus plant grown on a nutrient solution labeled with ^{45}Ca

A quantity of 0.48 ml sap was applied onto a paper strip and brought into the Beckman electrophoresis apparatus. As a comparison the same was done with a solution of $^{45}CaCl_2$ in water. As a buffer a mixture of 0.05 *M* HCl and 0.1 *M* tris (hydroxymethyl)aminomethane in water with a pH of 8.2 was used. The electrophoresis was carried out at 300 V for 2½ h. The paper was then cut into strips of ½ cm. After incineration the remaining ash was dissolved in 1 *M* hydrochloric acid and, after adding a scintillation liquid (Ringoet and De Zeeuw 1968), counted in a Philips scintillation counter PW 4510.

Results

1. *Observations on redistribution*

The results given in Table 1 reveal that the extent of redistribution from the leaf to the fruit in apple decreases from

Table 1. *Contents of potassium, magnesium, calcium and manganese in leaf and fruit in an experiment with different lime levels with Cox Orange Pippin.* Fruit samples were taken in the middle of September, leaf samples in the middle of August. Values are averages of the four lime levels.

Experimental year	Element	Content fruit: content leaf	Content, % of dry matter	
			in leaf	in fruit
1971	K	0.65 ± 0.03	1.32 ± 0.19	0.86 ± 0.04
	Mg	0.25 ± 0.05	0.19 ± 0.03	0.046 ± 0.000
	Ca	0.037 ± 0.006	1.16 ± 0.05	0.043 ± 0.004
	Mn	0.020 ± 0.004	0.016 ± 0.001	0.00030 ± 0.00004
1972	K	0.65 ± 0.05	1.29 ± 0.07	0.83 ± 0.03
	Mg	0.18 ± 0.03	0.21 ± 0.03	0.035 ± 0.002
	Ca	0.024 ± 0.003	1.36 ± 0.18	0.033 ± 0.001
	Mn	0.12 ± 0.09	0.0025 ± 0.0018	0.00029 ± 0.00001

potassium to magnesium, while, in this respect, calcium and manganese are even less mobile. As a standard for the extent of redistribution the ratio between the contents in the fruit and the leaf has been used. It has been assumed that the fruit is mainly supplied via the phloem, with organic material as well as minerals and water. In 1972 this ratio for manganese was only a little lower than that for magnesium. The manganese content in the leaf that year was very low so that for this reason a high relative transport can thus be explained. Especially at the higher lime treatments 11 mg/l Mn are clearly in the deficiency range. Based on these results, the elements studied can be put into the sequence potassium > magnesium > calcium and manganese of declining secondary mobility.

Figure 2 shows that, before leaf drop, calcium accumulates in the blade while its potassium content declines. This also indicates a greater potassium mobility.

2. *Contents and solubility*

The mineral contents of the examined samples of Ricinus and Yucca exudate are given in Table 2.

Qualitatively, the phloem sap of both species is similar especially with respect to their very low calcium and manganese contents.

The results of the determinations of precipitation on addition of the separate chlorides are given in Figure 3. It is evident that manganese addition to Yucca sap gives immediate precipitation, while in Ricinus sap the precipitation point is soon reached. An addition of calcium also induces a rapid precipitate, at smaller amounts in Yucca than in Ricinus. In Ricinus the precipitation occurs earlier when the plant has been cultivated on a nutrient solution with a higher Ca/K ratio. As a measure of the maximum possible transport in the sieve tube sap the total quantities of the different ions are calculated. From these it follows that the maximum concentrations in our sap samples are about 7 mg/l calcium and about 0.2 mg/l manganese for Yucca. For Ricinus we found about 15 mg/l calcium and 10 mg/l manganese for the plants cultivated on the 20/70 nutrient

Table 2. *Contents of potassium, magnesium, calcium and manganese in the sieve tube sap of Yucca and Ricinus* (mg/l). In Ricinus K: Ca = 20: 70 (except for value within parentheses 60:30).

Element	*Yucca*	*Ricinus*
K	1560	2680
Mg	62	106
Ca	5.5	3.4 (2.8)
Mn	0.2	0.3

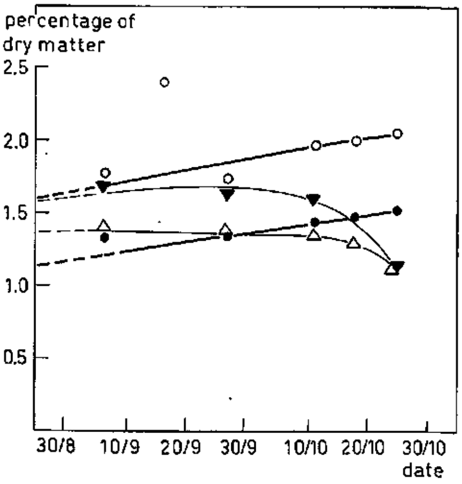

Figure 2. *Ca and K content in the blade of apple leaf* (Cox Orange Pippin) *during the last two months before leaf drop*. Experimental field 1 : Ca (○), K (△). Experimental field 2: Ca (●), K (▼).

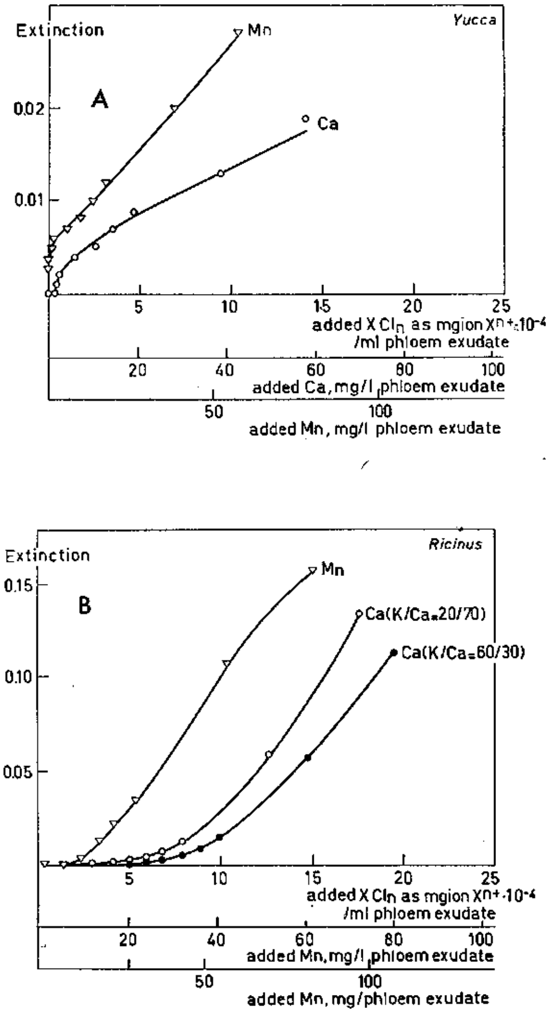

Figure 3. *Trend of extinction at 400 nm as a standard for the formation of precipitate in phloem sap of Yucca flaccida* (A) *and Ricinus communis* (B). The extinction was plotted against the quantity of added salt XCl_n. Ricinus was cultivated on a medium with K:Ca = 20:70 in equivalents and K:Ca = 60:30 in equivalents.

solution and about 22 mg/l calcium for the low calcium plants cultivated on the 60/30 solution.

When solutions of potassium chloride, magnesium chloride and lithium chloride were added, the limit of solubility was not reached. Not until much larger quantities of 3×10^{-3} mgion/ml sap were added a small precipitation occurred with potassium and magnesium in Yucca. When a barium chloride solution was added to Yucca sap, precipitation was observed after addition of amounts about equivalent to those of calcium chloride.

3. *Mode of occurrence of calcium in the phloem sap*

The *in vitro* experiments — not described in this publication — in which $^{45}CaCl_2$ was added to the exudate of Yucca gave an indication that originally present Ca reacted in about the same way as ionic ^{45}Ca. This could be concluded from the results of thin-layer chromatography and electrophoresis. The results of the *in vivo* experiment are given in Figure 4. Figure 4A gives the results of the scanning of the paperelectrophoresis strips of the phloem sap of a Ricinus plant provided with ^{45}Ca in the nutrient solution. It was evident that about 87% of the ^{45}Ca is detected at the same place as the ionic calcium (Figure 4B), while only 5% remains at the point of application.

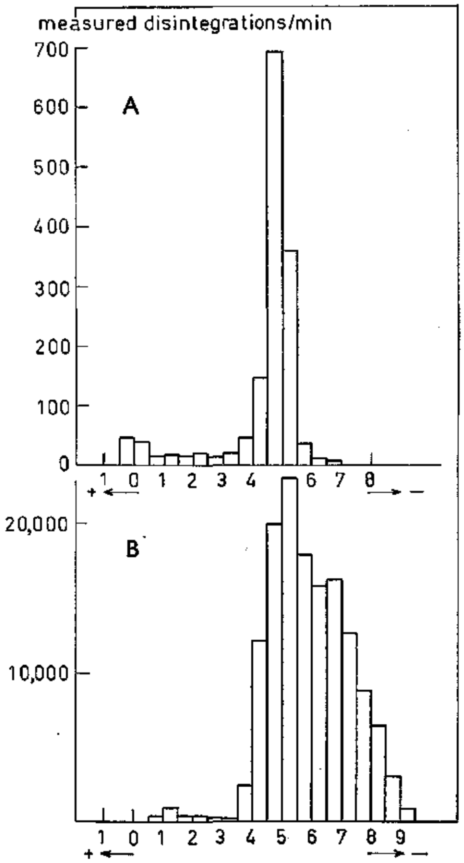

Figure 4. (A) *Paper electrophoresis of phloem sap of Ricinus after cultivating in a liquid medium supplied with* ^{45}Ca. (B) *Paper electrophoresis for a* $^{45}CaCl_2$ *solution.*

Discussion

The sequence of declining extent of redistribution from leaf to fruit, which we found for the cations, was potassium > magnesium > calcium ≈ manganese. It has been assumed that the fruit is mainly fed by the phloem (Wiersum 1966). Also the changes in the mineral leaf contents just before leaf drop indicate that potassium is much more mobile than calcium. The results obtained correspond to those in the literature (Price 1970, Crafts and Crisp 1971). Additionally it may be mentioned that some observations on magnesium and manganese mobility in other experiments depended on their concentration in the leaf. This type of phenomenon may account for the differences in the mobility ascribed to magnesium and manganese by different authors. As regards the ratio between contents in leaf and exudate, Tammes and Van Die (1966) observed the same sequence for the different elements in Yucca. It is now clear that elements of restricted mobility, such as calcium and manganese, only occur in the sieve tube sap in minor quantities. To explain these differences in content of the several ions, reference can be made to the three assumptions stated in the introduction. These possibilities have been more elaborately discussed elsewhere, *e.g.* Fischer (1967).

The first assumption, assuming differences in the active process of "vein-loading" for separate ions, was not examined in this investigation.

The possibility that differences in the degree of fixation to the parenchymatic tissues play a part, cannot be excluded, because it has been found that the strength with which ions are bound to a cation exchanger shows a similar sequence of barium > calcium > magnesium > alkali metals (Griessbach 1957).

Another possible explanation could be based on the solubility differences of the salts in watery solution. For the solubility of the oxalates, the sequence potassium > lithium > natrium ≫ barium ≈ magnesium > strontium ≫ calcium has been found and for the monohydrogen phosphates potassium > natrium ≫ lithium ≈ magnesium ≫ calcium ≫ barium. These sequences correspond more or less to those of the observed redistribution capacities. The greater relative mobility at low general plant content, *e.g.* in manganese, would be better explained by its solubility and the assumption of a maximum content in the phloem sap than by means of strong fixation in the surrounding tissues.

For the determination of the possible role of the solubility in regulating secundary mobility, it was important to investigate whether the element occurs as an ion or in a chelated condition. Only calcium was investigated in this respect. It was observed that the behaviour of ^{45}Ca in the sieve tube sap was not different from that of ionic ^{45}Ca in the chromatography and electrophoresis experiments. It thus

B.J. van Goor and D. Wiersma. Redistribution of Potassium, Calcium, Magnesium, and Manganese in the Plant. Physiol. Plant. 31 (1974) 163-168.

ERRATUM

page 168: the third reference should read:

Fischer, H. 1967. Phloemtransport und Stoffaufnahme. - Z. Pflanzenernähr. Bodenkd. 118: 100-111.

appears, that at least for calcium, the normal laws of solubility may be applied.

The solubility, which was demonstrated for both plants in the range of concentrations investigated, corresponds both to mobility data in the literature and to those found in our experiments with apples. The solubility observed was measured up to a concentration of 10^{-3} mmol added chloride per ml sap. No precipitate occurred with potassium and magnesium, while with addition of manganese immediate or very rapid precipitation resulted, and with calcium addition very rapid precipitation occurred soon after a small addition. The element barium, which gives a precipitation of about the same extent as calcium, is also hardly mobile in the plant (Bukovac and Wittwer 1957).

Summarising it can be stated that the solubility of the different elements in the sieve tube sap possibly play a part in combination with other mechanisms in determining the extent of redistribution of these elements in the plant. However, the possibility may not be excluded that differences in solubility already exert their influence in determining the composition of the symplasmatic continuum outside of the sieve tube cells.

A point which is still being investigated is the determination of solubility-restricting anions. Taking into account the amount of inorganic phosphate in the Yucca sap we investigated, only an amount of about 25 mg/l calcium could be present in solution without precipitation occurring, but this is more than actually observed. In preliminary tests it was found that addition of oxalate to the sap could restrict calcium solubility, while addition of phosphate only increases the amount of precipitate but does not induce earlier formation.

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