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THE ACTIVE ROLE OF THE HOST (VICIA FABA L.) IN THE TRANSFER OF NUTRIENT ELEMENTS FROM THE PHLOEM TO THE PARASITE (CUSCUTA SPECIES): METABOLICALLY CONTROLLED K^+ AND Mg^{++} RELEASE TO THE FREE SPACE

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SUMMARY

By washing out potassium and magnesium from the free space of stem segments of *Vicia faba*, parasitised stem parts were shown to have an efflux pattern different from that of normal ones.

Using the graphical method of compartmental analysis it could be shown that the K^+ and Mg^{++} efflux from parasitised stem parts does not occur in accordance with the regularities displayed by non-parasitised stem parts and known for other higher plant tissues.

The process causing the different efflux pattern is apparently under metabolic control since it appeared to be inhibited at 0 °C and after addition of 2,4-dinitrophenol or sodium azide. The possibility is discussed that the increased efflux is at least partly caused by an enhanced unloading rate for K^+ and Mg^{++} by the host phloem as was shown in a previous study for ^{14}C-solutes.

1. INTRODUCTION

The stem parasite *Cuscuta* usually has a very deleterious influence on the growth of the host by withdrawing nutrient elements and metabolites. Using ^{14}C-labelled metabolites it could be shown that *Cuscuta* is able to withdraw almost all assimilates which normally move from a photosynthesizing host leaf to growing fruits of *Vicia faba* (WOLSWINKEL 1974a).

A great enlargement of the surface area of the absorptive parts of the haustorial organ emerges as a feature seeming to be essential for the intensive transfer of solutes; a parallel can be found between the haustorium of *Cuscuta* and the absorptive epithelium of the small intestine of animals (WOLSWINKEL 1974a). In the parts of the wall adjacent to the sieve element of the host, the parasitic cell develops a conspicuous wall labyrinth (DÖRR 1968, 1972, KOLLMANN & DÖRR 1969), thus showing "transfer cell" characteristics (GUNNING & PATE 1969, PATE & GUNNING 1972.)

In the case of the absorptive hyphae of *Cuscuta* the function of the transfer cell can be related to absorption of solutes from an extracytoplasmic compartment. From the thin tissue cylinder of broad bean stem segments solutes present in the free space (the extracytoplasmic compartment) can be washed out with water. Using the method of washing out ^{14}C-solutes from the free space (cf.

KURSANOV & BROVCHENKO 1969, 1970) evidence has been presented that in addition to a very efficient absorption by the parasite, as suggested by the anatomical details, also an enhanced unloading rate of the host phloem is essential for the transfer of ^{14}C-metabolites from host to parasite (WOLSWINKEL 1974b). The assumed unloading of the host phloem is apparently under metabolic control since it appeared to be inhibited at 0° C and after addition of dinitrophenol.

If a membrane-mediated transfer of ^{14}C-solutes via the free space is essential for the intercellular transport in the contact region of host and parasite, an influence of *Cuscuta* upon the release of nutrient elements like potassium by the host stem tissues may be expected too. Potassium is the quantitatively most important cation in *Cuscuta* (unpublished data; cf. ANSIAUX 1958, SCHEIDECKER 1963). It is known to be a typically phloem-mobile element (e.g., CRAFTS & CRISP 1971, EPSTEIN 1972) and was shown to be present abundantly in broad bean stems, especially in parasitised stem parts (WOLSWINKEL 1973).

The present paper shows that a metabolically controlled release of K^+ and Mg^{++} by the host phloem is an important factor involved in the transfer from host to parasite.

2. MATERIAL AND METHODS

2.1 Cultivation of plants

Host and parasite were grown as described earlier (WOLSWINKEL 1974a). *Vicia faba* L. cv. Witkiem was used as host and three species of the genus *Cuscuta* were used as parasite, viz. *C. europaea* L. , *C. lupuliformis* Krocker, and *C. reflexa* Roxb. During the growing season of 1974 the plants were grown in the laboratory garden; the following winter the plants were grown on a nutrient solution in the greenhouse.

2.2 The procedure of washing out K^+ and Mg^{++} from the free space of hollow stem segments

Stem parts of *Vicia faba* were obtained as described in a previous paper (WOLSWINKEL 1974b).

The stem segments (about 4 cm in length) with or without haustorial coils (wound surfaces on the haustorial coil were covered with silicone rubber) were transferred to Erlenmeyer flasks of 300 ml containing demineralized water (25–50 ml, depending on the weight of the segments) and shaken 120 times per minute in a horizontal direction at 28° C. In some experiments with plants grown in the garden, Ca^{++} was added to the demineralized water (cf. LAÜCHLI & EPSTEIN 1970), although it had no distinct effect on the K^+ efflux pattern. In the experiments with the younger plants, grown in the greenhouse, always 0.5 mM $CaSO_4$ was added because this addition appeared to be necessary to obtain a regular pattern of Mg^{++} efflux.

After the first period of washing, permitting efflux of solutes from the tissues, the washing solutions were decanted and stored for K^+ and/or Mg^{++} analysis.

Subsequently, a second quantity of water was added to each flask, and this process was repeated several times. Initially solution changes were frequent, but thereafter the frequency was progressively reduced. After the last washing period the stem parts were stored for K^+ and/or Mg^{++} analysis. The amounts of the element in the series of washing solutions were added to the amounts remaining in the tissues, giving the amounts of the nutrient elements present in the tissues at the start of washing.

2.3 Potassium and magnesium analysis

When the washing procedure was finished, the haustorial coil was detached from the parasitised segments and its fresh weight subtracted from the weight of stem part and coil together, determined at the start of the experiment. In the experiments with *C. europaea* presented in this paper, it was usually only a few % of the combined weight. The dry weights of the stem parts were determined after drying at 105° C. The dry parts were placed in porcelain dishes and ashed in an electric furnace at about 570° C. The ash was dissolved in 3N hydrochloric acid and the solution obtained diluted with demineralized water. Potassium and magnesium in the washing solutions and in the ash were determined by atomic absorption spectroscopy using an air-acetylene flame and an Optica Densatomic (Milano) Atomic Absorption and Flame Emission Spectrophotometer.

2.4 Numbering of plant parts

In the figures internodes are numbered. The sign (u) after a numbered internode means the upper half of the internode is sampled, (1) means the lower half is sampled. Leaf 1 and 2 are the small reduced leaves at the stem base and leaf 3 is the first normal leaf. Usually the first flowers develop in the axil of leaf 7. The internode number corresponds to that of the leaf above it.

2.5 Presentation of results

Data of some typical experiments are presented. In spite of quantitative variations between the values of the amount of nutrient elements washed out during the series of washing periods (depending, e.g., on the vigour of the parasite, the stage of development of host and parasite, the growing conditions, the number of windings of the haustorial coil around the stem segment of the host, the stem part used, and the species of *Cuscuta*) the phenomenon described appeared to be reproducible.

In most figures, our data are presented as done by other authors using the technique of compartmental analysis in studies of higher plant tissues (PITMAN 1963, MACKLON & HIGINBOTHAM 1970, PIERCE & HIGINBOTHAM 1970, LORENZINI 1970, and HELLER et al. 1973 for monovalent cations and anions in higher plant tissues; KHOLODOVA 1967 for sucrose in sugar beet storage root tissue; GLINKA 1974 for thiourea in carrot storage root discs).

Using the efflux data, the logarithm of the amount of potassium or magnesium remaining in the tissue is plotted against time (*figs. 2, 4, 5,* and *6*). At longer

periods, in non-parasitised stem parts a steady loss results and we believe, in accordance with the view of the authors mentioned, that this represents loss from the vacuole, the slowest exchanging compartment. The resulting line has a slope which is the rate constant, k_v (^{10}log % potassium/time), for loss from the vacuole. Extrapolation of this straight line to zero time gives an intercept which represents the amount of potassium initially present in the vacuole. Subtraction of the amount of potassium in the vacuole from the amount of potassium in the tissue as a whole gives the amount of potassium in the remaining compartments believed to be the cytoplasm and the cell wall including intercellular spaces. Replotting again the logarithm of the remaining amount of potassium against time, yields a curve whose final straight section represents the loss of potassium from the cytoplasm (*fig. 3*). Extrapolation of this line to zero time gives an intercept which represents the amount of potassium initially present in the cytoplasm. Finally, the amount of potassium in the free space (cell wall and intercellular spaces and lumen of cells wounded during sampling), which is released very fast, is represented by the amount of potassium in the total tissue less the amounts in cytoplasm and vacuole.

3. RESULTS

Fig. 1 shows results of a typical experiment with a broad bean stem parasitised by *C. europaea*. Efflux of K^+ was measured over a period of 3 hr. In the first 10 min, between 1 and 2% is found in the washing solutions of the four stem parts. In the four subsequent periods for all stem parts values below 1% are found.

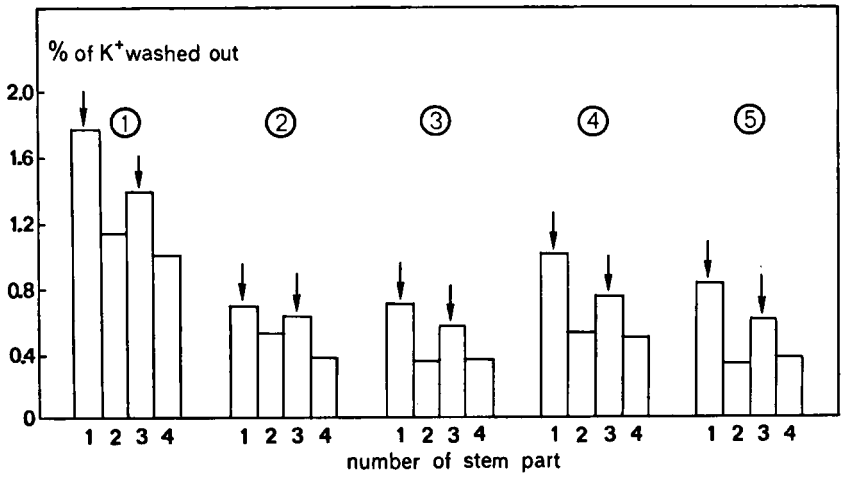

Fig. 1. The amounts of K^+, washed out into demineralized water during 5 successive periods of resp. 10, 20, 30, 60, and 60 min. Host parasitised by *C. europaea* at int. 6 and int. 10. Parasitised internodes indicated by arrows. Number 1 represents int. 6, number 2 represents int. 7, 8, and 9, number 3 represents int. 10, and number 4 represents int. 11.

In parasitised stem parts more K^+ is released to the free space than in non-parasitised ones. The differences between both types of stem parts are more conspicuous in the last periods. In the series of first to fifth period the average of the values (indicating % K^+ washed out) of the two parasitised stem parts is 48%, 46%, 78%, 74%, and 109% higher respectively than the average of the values of the two non-parasitised stem parts. Apparently, the rate of K^+efflux decreases with time much faster in non-parasitised stem parts than in parasitised ones. Similar results were obtained in many other experiments. An important fact seemed the observation that this sharp difference between parasitised and non-parasitised stem parts disappeared when the experiments were done at 0° C.

In order to obtain a more detailed picture of the phenomenon involved the graphical method of compartmental analysis (references mentioned in 2.5) was introduced. It appeared necessary to follow the efflux pattern over more than 2 or 3 hr. Most references, when the amount of K^+ remaining in the tissue is plotted logarithmically against time, show the graph to become a straight line after about 2 hr. Therefore we used a standard procedure of washing for at least 5 or 6 hr.

Fig. 2 shows results of an experiment with a stem, parasitised by intensively growing *C. europaea*. Non-parasitised stem parts show an efflux pattern completely comparable with data presented by other authors, using the method of compartmental analysis mentioned in 2.5. After about 2 hr the efflux curve becomes linear. In many other experiments, in which solution changes were more frequent in the period between 2 and 6 hr after the start of washing, similar results were obtained. In *fig. 2* the line through the two points, corresponding with the amount of K^+ remaining in the tissue after 5 and 6 hr respectively, has been extrapolated to zero time. As shown, the graph is already almost linear after 2 hr. The linear parts of the curves 1, 3, and 4 have the same slope, indicating that the rate constant for loss from the vacuole is equal in all non-parasitised stem parts used. Apparently, younger stem parts are characterised by a higher efflux rate in the first 2 hr than in older stem parts. As is shown in *fig. 3*, this is caused by a higher amount of K^+ initially present in the cytoplasmic phase.

Curve 2, representing the behaviour of the parasitised stem part, is different from the other three curves. In the parasitised stem part there is a higher K^+ efflux rate, as was also shown in *fig. 1*. Besides, if the line through the 5 and 6 hr points is extrapolated to zero time, the graph deviates much from being almost linear after 2 hr. In many other experiments with more solution changes in the last 4 hr it was confirmed that the curve of parasitised stem parts never becomes linear during the last hours of the experiment.

Fig. 3 shows efflux of K^+ from cytoplasm and free space of the same non-parasitised stem parts as were shown in *fig. 2*. The curves have been obtained by subtracting the amount of K^+ in the vacuole from the amount of K^+ in the tissue as a whole. Efflux of K^+ from cytoplasm and free space of broad bean stem segments is completely comparable with the pattern, known for other tissues in literature. Extrapolation of the final linear part of the curve gives an intercept

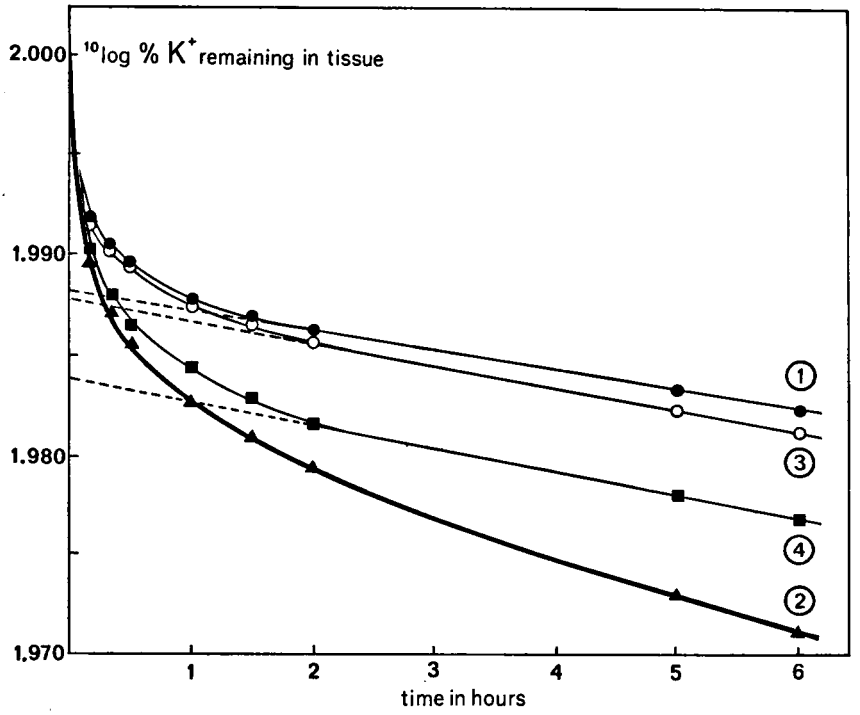

Fig. 2. Amounts of K^+ in stem parts plotted on a logarithmic scale, as a function of time, during the course of efflux into water. The broken lines indicate extrapolation of the amounts of K^+ in the slowest exchanging compartment (the vacuole) to zero time. Host parasitised by *C. europaea* at lower part of int. 6. Number 1 represents int. 5 (u), 2 represent int. 6 (1), 3 represents int. 6 (u), and 4 represents int. 7.

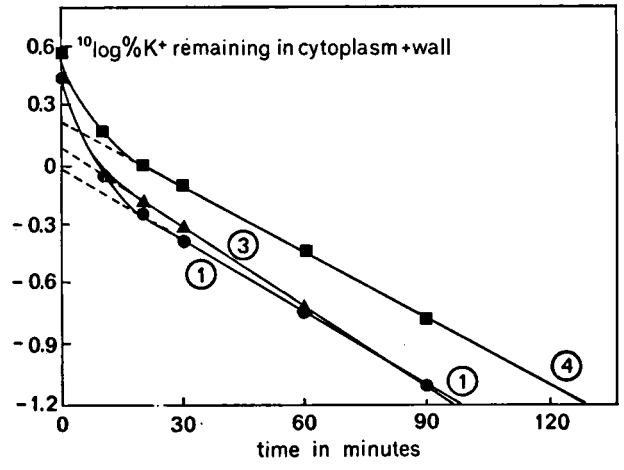

Fig. 3. Amounts of K^+ in the same stem parts as shown in *fig.* 2 (stem part 2, however, not shown), after subtracting the amount of K^+ in the vacuole, as a function of time, during the course of efflux into water. The broken lines indicate extrapolations of the amount of K^+ in the cytoplasm.

which represents the amount of potassium initially present in the cytoplasm. As can be seen in the figure, efflux from the free space has finished after the first 30 min.

Tentatively, a curve was also drawn for the parasitised stem part (not presented in *fig. 3*) after subtracting the amount of K^+ supposed to be in the vacuole from the amount of K^+ in the tissue as a whole. The amount of K^+ in the vacuole was derived from extrapolation of the line through the 5 and 6 hr points to zero time. This proved to be a correct procedure for the non-parasitised parts of the parasitised stem. As expected, curve 2 deviated strongly from the pattern shown by curves 1, 3, and 4. In the first hour the curve most resembled curve 4, but during the second hour the curve became irregular and showed a slower decline than the linear parts of the curves of the non-parasitised stem parts. In this way it has been demonstrated in other experiments too that the method of compartmental analysis cannot be used as in non-parasitised stem parts. Efflux from parasitised stem parts after the first 2 hr seems to be more complicated than efflux from the vacuole alone.

Regularly it was found that non-parasitised stem parts situated adjacent to heavily parasitised ones also showed the changed K^+ efflux pattern characteristic of the latter, quite comparable to similar observations on ^{14}C-efflux (WOLSWINKEL 1974b). Apparently the altered physiological conditions of the parasitised stem parts, which cause enhanced efflux, not only exist at the site of attachment of the parasite, but regularly also in adjacent regions.

Fig. 4 presents results of a typical experiment in which K^+ efflux was studied at 0° C. For this experiment a young plant was used, grown in the garden late in the season. Non-parasitised stem parts of young plants do not all exhibit exactly the same efflux rate from the vacuole in the last hours of the efflux experiments, as was shown commonly to occur in older plants, decapitated earlier in the season. The slope of the linear part of the curves 1 and 3 is not completely the same; often younger internodes exhibit a steeper slope.

As can be seen in *fig. 4*, curve 2 shows a linear part during the last hours of efflux in the same way as shown by non-parasitised stem parts. The phenomenon at normal temperature causing a more complicated efflux pattern than efflux from the vacuole alone, has completely disappeared. During the first 120 min curve 2 has a form distinctly different from that of curves 1 and 3. Efflux from the cytoplasmic compartment is more important in the parasitised stem part suggesting a more important role of the cytoplasmic phase in parasitised parts. In this context, also the high levels of P and Mg in parasitised stem parts may be mentioned, also found normally in the fast growing top parts too of broad bean stems (WOLSWINKEL 1973), which can be related with a more intensive metabolism at those places. After subtraction of the amount of K^+ in the vacuole, for all three stem parts curves are obtained for efflux of K^+ from cytoplasm + free space, which have a final linear part after the first 30 min (cf. *fig. 3*). Initially, the parasitised stem part is characterized by the highest amount of K^+ in cytoplasm, but the final linear part of curve 2 has practically the same slope as curve 1, indicating that efflux from the cytoplasm is similar to that in control segments. The influence

of the parasite can not longer be found in the efflux pattern. Apparently, the phenomenon studied is completely inhibited at 0°C.

When 10^{-4}M or 5.10^{-5}M 2,4-dinitrophenol (DNP) were added to the washing water an abnormally high K^+ efflux in both parasitised an non-parasitised stem parts was demonstrated, indicating damage to the permeability barriers within the tissue. The harmful effect of the high DNP concentrations became clear already within 30 min (cf. MARETZKI & THOM 1972, who observed a similar influence of 10^{-4}M DNP on the efflux of sugars). 10^{-6}M DNP did not cause such effects as demonstrated by a normal efflux rate from non-parasitised stem parts over a period of 6 hrs. In a number of experiments in which a set of parasitised plants of the same age was used, the deviation from the normal efflux pattern appeared to be smaller when efflux was measured in 10^{-6}M DNP than when efflux was measured in water. When 2.10^{-6}M or 5.10^{-6}M were used, hardly any influence of *Cuscuta* could be found; no harmful effect of this concentration could be found as was observed for the higher concentrations mentioned above. If 10^{-5}M DNP was used, abnormal K^+ efflux sometimes started to occur after a few hours, progressively increasing with time. The youngest internodes of the stem part used appeared most sensitive to the harmful influence since in them abnormal leaking started first and increased fastest with time. A clear influence of *Cuscuta* could no longer be found when 10^{-5}M DNP was used.

Fig. 5 shows results of an experiment in which 5.10^{-5}M NaN_3 was added to the washing water. Efflux from the cytoplasm and the free space from the non-parasitised stem parts (curve 1 and 3) of this young plant appears to have finished after about $3\frac{1}{2}$ hr. For, if the line through the 4 and 5 hr points is extrapolated to zero time and the amount of K^+, assumed to be in the vacuole, is substracted, the efflux curves 1 and 3 for efflux from cytoplasm + free space can be found to become linear after 30 min. This observation supplies evidence that after about 3 hr only the vacuole still contributes to the efflux from the tissue. When the same procedure is applied to curve 2, a curve for efflux from cytoplasm + free space is found not to become completely linear after 30 min, although the deviation from linearity is much smaller than found for curve 2 of *fig. 2*. Apparently, the enhanced efflux, caused by *Cuscuta*, is inhibited strongly although not completely by 5.10^{-5}M NaN_3.

The concentrations of DNP and NaN_3, causing complete or considerable inhibition of enhanced efflux, seem comparable with the values found by other investigators who studied the effect on metabolically controlled processes in plant tissues, e.g. the effect on amino acid uptake by higher plant cells (CHEUNG & NOBEL 1973, KING & OLENIUK 1973).

Fig. 6 shows results of an experiment in which Mg^{++} efflux was measured. Int. 7 (curve 3) of the host was parasitised by *C. europaea*. A strong parallel can be found with the above mentioned influence of *Cuscuta* on the K^+ efflux. Comparable results were found in other experiments too.

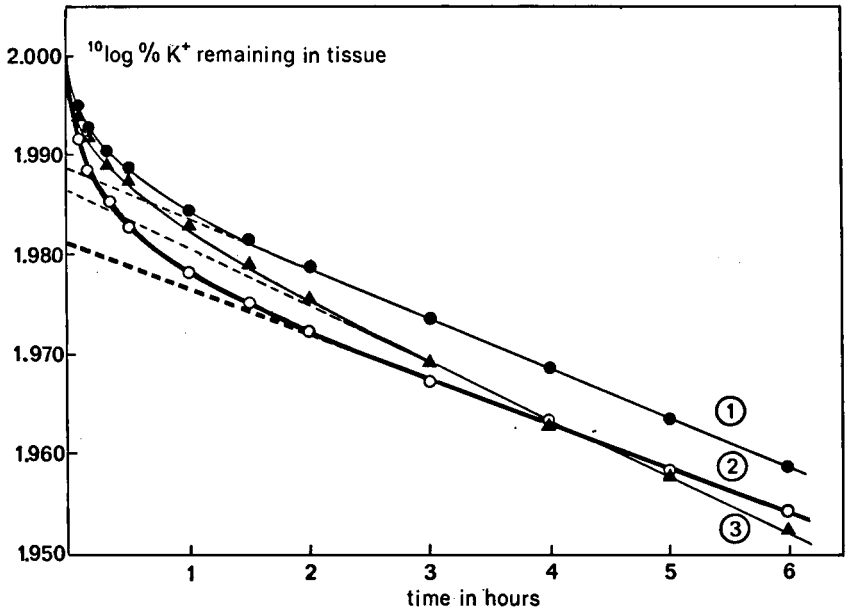

Fig. 4. Amounts of K^+ in stem parts plotted on a logarithmic scale, as a function of time, during the course of efflux into water at 0 °C. The broken lines indicate extrapolation of the amounts of K^+ in the vacuole to zero time. Host parasitised by *C. europaea* at int. 6. Number 1 represents int. 5, number 2 represents int. 6, and number 3 represents int. 8 and 9.

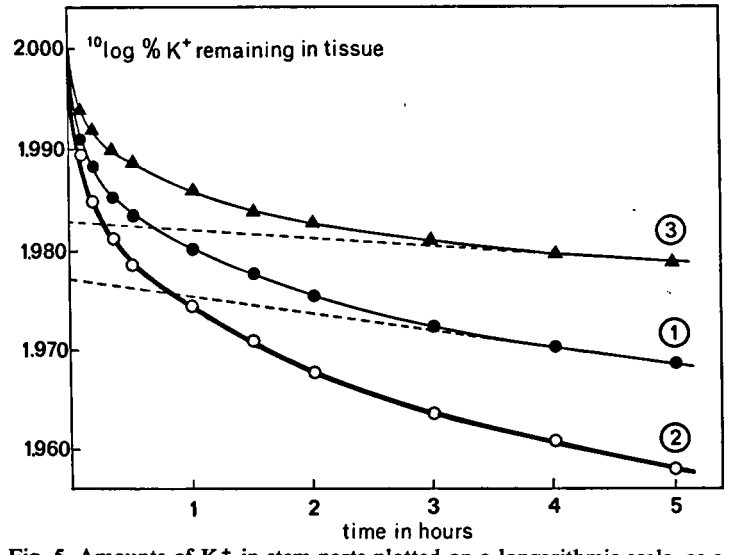

Fig. 5. Amounts of K^+ in stem parts plotted on a longarithmic scale, as a function of time, during the course of efflux into 5.10^{-5}M NaN_3. The broken lines indicate extrapolation of the amounts of K^+ in the vacuole to zero time. Host parasitised by *C. europaea* at int. 6. Number 1 represents int. 5, number 2 represent int. 6, and number 3 represents int. 7.

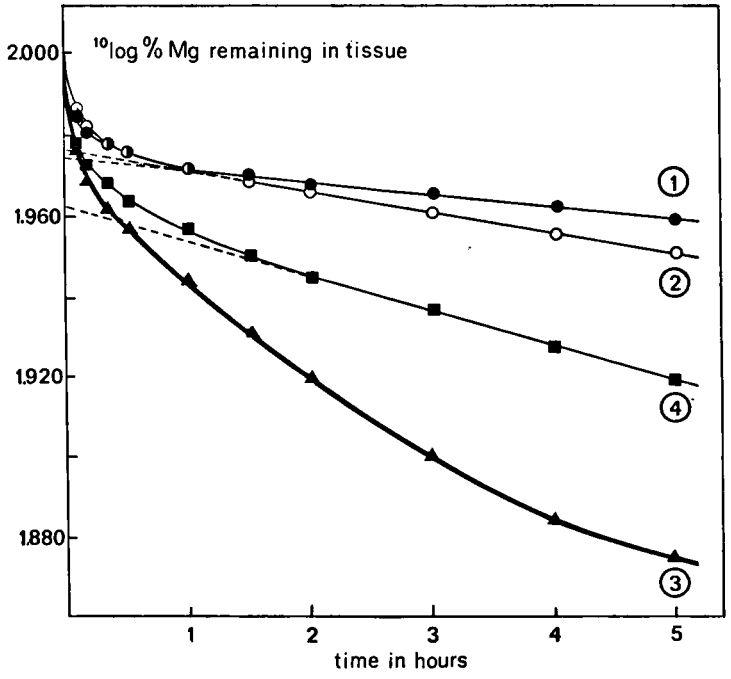

Fig. 6. Amounts of Mg^{++} in stem parts plotted on a logarithmic scale, as a function of time, during the course of efflux into 0.5 mM $CaSO_4$. The broken lines indicate extrapolation of the amounts of Mg^{++} in the vacuole to zero time. Host parasitised by *C. europaea* at int. 7. Number 1 represents int. 3 and 4, number 2 represents int. 5 and 6, number 3 represents int. 7, and number 4 represents int. 10 and 11.

4. DISCUSSION

The efflux pattern of Mg^{++} from broad bean stem parts is in accordance with the regularities reported in literature for monovalent ions, and is comparable to that of K^+ in our experiments. Considerable quantitative differences, however, exist as shown in *fig. 6*. In control experiments too it was demonstrated that the Mg^{++} efflux rate is relatively higher than the K^+ efflux, as shown by the higher percentages released to the free space.

This may have several reasons:

a) A higher percentage of the Mg^{++}, initially present, is localised within the cytoplasmic compartment. Whereas it was shown in many experiments that only less than 5% of K^+ was present in the cytoplasm, in several experiments values of about 10% were found for Mg^{++}. This may result in a relatively higher efflux during the first 120 min.

b) The rate constant for Mg^{++} efflux from the vacuole is much larger (in many experiments about 5–10 × more) than the rate constant found for K^+.

The Mg^{++} efflux experiments supply data of an important divalent cation, not studied by the authors mentioned in 2.5.

Our experiments on the efflux of K^+ and Mg^{++} from stem segments indicate a change in the permeability of stem tissues after infection by *Cuscuta* as demonstrated by the changed efflux pattern of these nutrient elements. This phenomenon seems comparable with that found in experiments on the release of ^{14}C-assimilates by the host phloem (WOLSWINKEL 1974b). In both situations a metabolically controlled enhanced release of phloem-mobile solutes to the free space has been found in parasitised stem parts.

The living cells of stem tissues can be divided into two groups:

a) elements directly or indirectly involved in longitudinal assimilate translocation: sieve tubes and companion cells.
b) the other cells: mainly parenchyma cells (including the outermost cell layers of the stem too like epidermis and collenchyma).

Recent data on the osmotic pressure of the mature sieve element-companion cell complex in source leaf, path, and sink leaf tissue of sugar beet supply new evidence for the view that these tissue elements have special qualities in comparison with parenchyma cells. The sieve element-companion cell complex is characterized by an osmotic pressure much higher than the mesophyll of source and sink leaves (GEIGER et al. 1973, FELLOWS & GEIGER 1974). The membrane of the sieve element-companion cell complex apparently forms a sharp demarcation line between the contents of this complex and its apoplast with the surrounding mesophyll cells on the other hand.

Several arguments are in favour of the view that the phenomenon, studied in this paper, is caused by the sieve element-companion cell complex.

1. A strong parallel does exist between K^+ and assimilates:

a) Both are typically phloem-mobile and show redistribution after primary transport, e.g. from leaves downwards. Typical sinks like fruits and *Cuscuta* show a high capacity to accumulate K^+ and assimilates.
b) The way in which considerable amounts of potassium, magnesium, and phosphorus are withdrawn from the host, is similar to the way ^{14}C-assimilates exported by a host leaf are drained by *Cuscuta* (WOLSWINKEL 1974a). Mineral analyses of broad bean leaves show a large decrease of the content of these nutrient elements in the leaves of a parasitised host after *Cuscuta* has started luxuriant growth (Wolswinkel, unpublished data). Comparable results were obtained by SINGH et al. (1971) and ABOU-RAYA et al. (1973) for broad bean and other host plant species, parasitised by the root parasite *Orobanche*. Whereas the mineral analyses of host tissues indicate an intensive export of these nutrient elements from the leaves, the fast growing tissues of *Cuscuta* are characterised by a high content of these elements.

2. If the direction and the intensity of the translocation of K^+, Mg^{++}, and assimilates are comparable, it may be expected that the mechanism of the transfer of these phloem-mobile nutrient elements is comparable with that of assimilates too. After evidence has been presented that a metabolically controlled enhanced release of assimilates occurs to the free space by the phloem (WOLSWINKEL 1974b), seeming to be a prerequisite for the complete transfer from host to parasite (WOLSWINKEL 1974a) by preceding the intensive absorp-

tion by the transfer cells of *Cuscuta*, in the present experiments a similar metabolically controlled enhanced release has been shown to exist for K^+ and Mg^{++}.

3. The inhibition of the increased release of K^+ and Mg^{++} by low temperature or metabolic inhibitors, found in the present experiments, is comparable with the inhibition of the enhanced ^{14}C-solute release in experiments, in which ^{14}C was localised mainly in phloem tissues.

Several arguments can be raised against the view that parenchyma cells play a quantitatively significant role:

1. In all the experiments with non-parasitised stem segments (with the exception of the youngest internodes of young plants) efflux of K^+ from free space and cytoplasm has finished after about 2–3 hr. Efflux from the cytoplasm should have finished too in parasitised segments after about 2–3 hr.
2. K^+ efflux from non-parasitised segments (mainly consisting of parenchyma cells) is not clearly influenced by low temperatures or metabolic inhibitors, whereas in experiments with ^{14}C-solutes it has been demonstrated that release by the phloem is very sensitive to these factors. Efflux of K^+ from the cytoplasm and vacuole of parenchyma cells seems not to be metabolically controlled and therefore the sites of the metabolically controlled and parasite induced enhanced K^+ efflux are not inside parenchyma cells.
3. Parenchyma cells of parasitised stem parts are not "a little bit leaky" when compared with cells in non-parasitised stem parts, as is known in tissues influenced by fungal toxins. On the contrary, they contain more solutes than cells in non-parasitised stem parts (WOLSWINKEL 1973). Because stem segments consist mainly of parenchyma cells, the enhanced nutrient level found is a characteristic of these cells too. They take advantage of the supply of nutrient elements to the free space of the stem tissue by absorbing a part of the solutes released to the free space under influence of *Cuscuta*. Another indication that parenchyma cells in parasitised stem parts do not exhibit a higher K^+ efflux rate than cells in non-parasitised parts comes from the observation that in the last stage of development of host and parasite parasitised stem parts show a smaller K^+ release to the free space than non-parasitised parts of the stem (Wolswinkel, unpublished data). Leakage of solutes seems to be characteristic of senescing tissues (e.g. SIMON 1974). The membranes of the tissues of the non-parasitised internodia of the dying host plant become leaky for nutrients, those in parasitised stem parts less so.

The data presented in this paper point to an important factor in the regulation of the radial translocation of phloem-mobile nutrient elements in the host-parasite combination, viz. metabolically controlled release to the free space of the tissue at the site where the parasite is attached.

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REFERENCES

ABOU-RAYA, M. A., A. F. RADI & M. M. DARWISH HEIKAL (1973): Host parasite relationship of Orobanche species. *Proc. Eur. Weed. Res. Coun. Symp. Parasitic Weeds*: 167–176. Malta University Press.

ANSIAUX, J. R. (1958): Sur l'alimentation minérale des phanérogames parasites. *Bull. Acad. roy. Belgique, Cl. Sci.*, 5 sér. **44**: 787–793.

CHEUNG, Y.-N. S. & P. S. NOBEL (1973): Amino acid uptake by pea leaf fragments. *Plant Physiol.* **52**: 633–637.

CRAFTS, A. S. & C. E. CRISP (1971): *Phloem transport in plants.* Freeman and Co, San Francisco.

DÖRR, I. (1968): Zur Lokalisierung von Zellkontakten zwischen Cuscuta odorata und verschiedenen höheren Wirtspflanzen. *Protoplasma* **65**: 435–448.

— (1972): Der Anschluss der Cuscuta-Hyphen an die Siebröhren ihrer Wirtspflanzen. *Protoplasma* **75**: 167–184.

EPSTEIN, E. (1972): *Mineral nutrition of plants: principles and perspectives.* John Wiley and Sons, Inc., New York-London-Sydney-Toronto.

FELLOWS, R. J. & D. R. GEIGER (1974): Structural and physiological changes in sugar beet leaves during sink to source conversion. *Plant Physiol.* **54**: 877–885.

GEIGER, D. R., R. T. GIAQUINTA, S. A. SOVONICK & R. J. FELLOWS (1973): Solute distribution in sugar beet leaves in relation to phloem loading and translocation. *Plant Physiol.* **52**: 585–589.

GLINKA, Z. (1974): Fluxes of a nonelectrolyte and compartmentation in cells of carrot root tissue. *Plant Physiol.* **53**: 307–311.

GUNNING, B. E. S. & J. S. PATE (1969): "Transfer cells"- Plant cells with wall ingrowths, specialised in relation to short distance transport of solutes – their occurrence, structure and development. *Protoplasma* **68**: 107–133.

HELLER, R., C. CRIGNON & D. SCHEIDECKER (1973): Study of the efflux and the influx of potassium in cell suspensions of Acer pseudoplatanus and leaf fragments of Hedera canariensis, in: W. P. ANDERSON, *Ion Transport in Plants*: 337–353. Academic Press, London-New York.

KHOLODOVA, V. P. (1967): Localization of sucrose in tissues of the storage root of the sugar beet. *Soviet Plant Physiol.* **14**: 367–381.

KING, J. & F. H. OLENIUK (1973): The uptake of alanine-^{14}C by soybean root cells grown in sterile suspension culture. *Can. J. Bot.* **51**: 1109–1114.

KOLLMANN, R. & I. DÖRR (1969): Strukturelle Grundlagen des zwischenzelligen Stoffaustausches. *Ber. dtsch. bot. Ges.* **82**: 415–425.

KURSANOV, A. L. & M. I. BROVCHENKO (1969): Free space as an intermediate zone between photosynthesizing and conducting cells of leaves. *Soviet Plant Physiol.* **16**: 801–807.

— & — (1970): Sugars in the free space of leaf plates: their origin and possible involvement in transport. *Can. J. Bot.* **48**: 1243–1250.

LAÜCHLI, A. & EPSTEIN (1970): Transport of potassium and rubidium in plant roots – the significance of calcium. *Plant Physiol.* **45**: 639–641.

LORENZINI, M. (1970): L'exsorption de K^+ par des disques frais de pommes de terre: compartmentation, action du Ca^{2+} et du Mg^{2+}. *Physiol. Vég.* **8**: 159–172.

MACKLON, A. E. S. & N. HIGINBOTHAM (1970): Active and passive transport of potassium in cells of excised pea epicotyls. *Plant Physiol.* **45**: 133–138.

MARETZKI, A. & M. THOM (1972): Membrane transport of sugars in cell suspensions of sugarcane. I. Evidence for sites and specificity. *Plant Physiol.* **49**: 177–182.

PATE, J. S. & B. E. S. GUNNING (1972): Transfer cells. *Annu. Rev. Plant Physiol.* **23**: 173–196.

PIERCE, W. S. & N. HIGINBOTHAM (1970): Compartments and fluxes of K^+, Na^+ and Cl^- in Avena coleoptile cells. *Plant Physiol.* **46**: 666–673.

PITMAN, M. G. (1963): The determination of the salt relations of the cytoplasmic phase in cells of beetroot tissue. *Aust. J. Biol. Sci.* **16**: 647–668.

SCHEIDECKER, D. (1963): La nutrition minérale des phanérogames parasites et des greffes. Intérêt de ces plantes comme matériel de l'étude. *Année biol. (Paris), sér.* **4, 2**: 307–336.

SIMON, E. W. (1974): Phospholipids and plant membrane permeability. *New Phytol.* **73**: 377–420.

SINGH, J. N., J. N. SINGH & T. B. RAI (1971): Studies on the physiology of host-parasite relationship in Orobanche. II. Growth and mineral nutrition of host and parasite. *Physiol. Plant.* **25**: 425–431.

WOLSWINKEL, P. (1973): The disturbance of the development of broad bean (Vicia faba L.) and the setting and growth of pods after infection by Cuscuta: experiments about translocation of assimilates. *Proc. Eur. Weed Res. Coun. Symp. Parasitic Weeds*: 177–187. Malta University Press.

— (1974a): Complete inhibition of setting and growth of fruits of Vicia faba L., resulting from the draining of the phloem system by Cuscuta species. *Acta Bot. Neerl.* **23**: 48–60.

— (1974b): Enhanced rate of ^{14}C-solute release to the free space by the phloem of Vicia faba stems parasitised by Cuscuta. *Acta Bot. Neerl.* **23**: 177–188.