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THE INFLUENCE OF LIGHT ON THE LOSS OF LABELLED PHOSPHORUS FROM BEAN LEAVES

BY

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INTRODUCTION

One advantage of using labelled substances is that with the aid of the appropriate apparatus the course of uptake and loss by plant organs can easily be traced with a single intact plant. However, in the field of plant physiology only a few such *in vivo* experiments have been performed.

In 1940 BREWER and BRAMLEY reported some results on P and Na uptake with maize plants. They studied the influence of light on uptake and loss by leaves and found striking results.

The influence of light on loss of substances from leaves is of particular interest in relation to the transport mechanism. It has been proved that the downward transport of 2,4-D from bean leaves only occurs if there is a simultaneous transport of sugar (WEINTRAUB and BROWN 1950, MITCHELL 1951). This fact is considered to be a proof for the presence of a mass flow as is assumed in Münch's theory on transport in sieve tubes.

Therefore, in the experiments reported here, the influence of light on the loss of labelled phosphorus, previously accumulated in leaves of bean plants, was studied.

Using Martin's continuous recorder (MARTIN 1952) in the Department of Agriculture, Oxford, experiments were started by the first author at the instigation of Dr. R. Scott Russell. Significant though slight effects, owing to the short experimental periods, were obtained. These investigations were continued in the Botanical Laboratory, Groningen, using longer experimental periods. Here only the results of these long-term experiments will be briefly discussed. It is realised that a satisfactory analysis will not be possible until much more work has been done.

MATERIAL AND METHODS

Intact bean plants (*Phaseolus vulgaris*), grown on a complete aerated Hoagland solution were used at an early stage of development, the primary leaves being fully expanded, while the first trifoliate leaf showed incipient unfolding.

The pretreatment was given in a constant-temperature room (20° C), in which also the experiments were performed. Fluorescent light was used for illumination.

Individual bean plants were placed in a Hoagland solution with labelled phosphorus (15 mg P/liter, 15–25 micro Curie P^{32}/liter), the period of absorption being dependent on the rate of absorption and accumulation in the leaf. The figures show that the final activity varied greatly between experiments. Redistribution was then studied while the plant was in a minus-P solution.

The course of the labelled phosphorus content of one of the opposite leaves was traced by placing a counter tube on the underside of the leaf. Philips counting equipment PW 4020, GM 4810 with counter tube 18513 (effective area 0.3 cm^2) was used.

Experiments

Figure 1A shows the results obtained with a young bean plant, which was allowed to absorb labelled phosphorus from a Hoagland solution for a 10 hours period. It was then placed on a Hoagland minus phosphorus solution for the rest of the experiment. The increase of the activity of one of the opposite leaves was studied. This leaf was kept in the light during the first 5–6 days, the other aerial parts being kept in the dark as schematically indicated at the top of the figure. The activity continued to increase for another three days, although the roots were placed in a minus-P solution. A maximum value was reached on the third day after which a rapid fall of the labelled phosphorus content could be observed. This fall continued to occur during the first two days in the following period, in which the whole plant was in the dark. But it was gradually changed into an increase. When, on the 7th day, the lowest value was reached, the total loss amounted to 10–12 % of the maximum value obtained on the third day of the experiment.

The leaf being placed once more in continuous light, a slight decrease could be observed during the first few days, but then hardly any further decrease occurred. This apparent inability to show any back transport whatsoever, was always observed at the end of these long-term experiments. The slow deterioration of the leaves is believed to explain this effect. This stresses the fact, that the losses observed depended on the metabolic activity of healthy leaves.

Figure 1B shows the results of a similar experiment in which the whole plant was in continuous light, except for one of the opposite leaves, the phosphorus content of which was traced, and which was kept alternately in the light and in the dark for some days as indicated in the figure. Absorption was slow; consequently the absorption period was extended to 3 days. A maximum value for the labelled phosphorus content was then reached. During the redistribution period the plant was on a minus-P solution. The pattern of changes was in accordance with that of the previous experiment.

Fig. 2 shows the results of an experiment in which the treatment

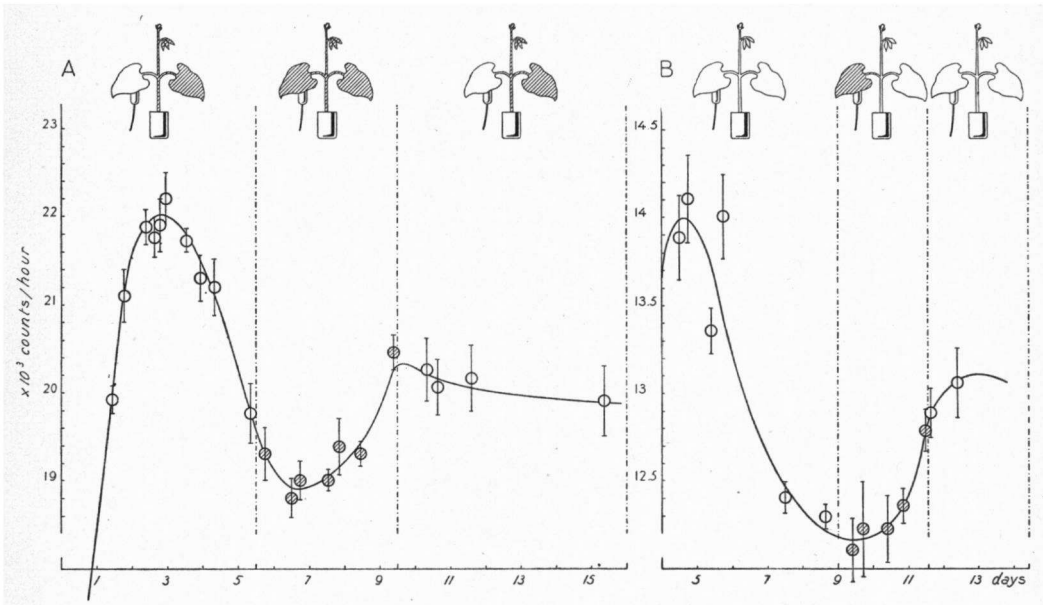

Fig. 1. The influence of light and dark on the redistribution of labelled phosphorus from a bean leaf. The bean plant was allowed to absorb a definite amount of labelled phosphorus from a Hoagland solution. Redistribution took place while the plant was in a phosphorus-free nutrient solution. During the redistribution period the plants were kept either in continuous light (B) or in continuous dark (A) exept for one of the primary leaves which was placed alternately in the light and the dark as shown at the top of the figure.
The labelled phosphorus content of the experimental leaf was determined by means of a Geiger-Müller tube, directly applied to the leaf, and is expressed in activity units. No importance should be attached to the differences in activity level between various experiments as they are mainly due to a variety of irrelevant factors like specific activity of the solution, position of the counting tube, the length of the absorption period etc. Finally, it should be realized that the fluctuations in labelled phosphorus content are small compared with the total amount of labelled phosphorus present in the leaf.

applied to the plant was in a way the reverse of the treatments in the previous ones.

The plant used was at a somewhat older stage; the first trifoliate leaf was very well developed, the size of the second trifoliate leaf being about 34 mm. The two opposite leaves were in a perfect condition and it was possible to extend the duration of the experiment, which lasted for almost 4 weeks.

This plant was allowed to absorb labelled P from a Hoagland solution for 47 hours, the whole plant being kept in the light. At the end of this period the plant was again transferred to a minus-P solution. During this period as well as during the following redistribution period the activity of one of the opposite leaves was studied. This leaf remained in the light, the other parts of the shoot being kept either in the light or in the dark. The labelled P content continued to increase for a while after the plant was put into minus-P

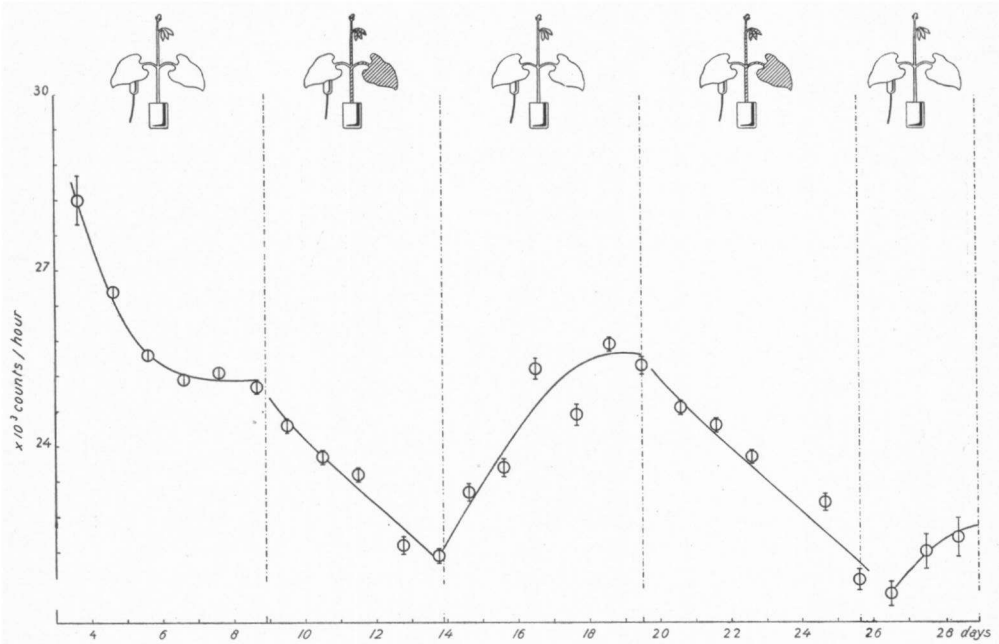

Fig. 2. The influence of the light conditions on the redistribution of labelled phosphorus from a bean leaf. During the redistribution period the plant was in a phosphorus-free nutrient solution. The light-dark treatments are schematically shown at the top of the figure: the experimental leaf was in continuous light, the other parts being either in the light or in the dark.

solution. A maximum value was soon reached and then a gradual decrease could be observed until after 5–6 days a more or less steady state was obtained. Then the greater part of the plant was placed in the dark. A further decline was induced which continued until the end of this dark period. But as soon as the whole plant was replaced in the light this fall was changed into an equally rapid increase of the labelled P content. After 4–6 days about the same activity-level was reached as at the end of the first light period. The perfect condition of this plant made it possible to repeat this dark and light treatment, and to confirm the results so far obtained, although during this second part of the experiment the trifoliate leaves showed an incipient colour changing so that, finally, the experiment had to be cut short.

Discussion

The loss of labelled phosphorus from a leaf, pretreated in such a way that its initial phosphorus content was high, was found to be promoted by illumination of this leaf. However, such a loss could only be observed a few days after the plant was transferred from a labelled nutrient solution to a minus-phosphorus solution. Initially the increase in labelled phosphorus content continued for a few days until a maximum was reached. This increase must be due to a continuation of the

supply from the roots. The fact that this initial increase in labelled phosphorus content was gradually changed into a fall suggests that the actual labelled phosphorus content of the leaf is at any time the result of an influx into and an efflux from the leaf. At any rate, in the opinion of the present authors the effects obtained in these experiments by the various light and dark treatments, can most readily be explained by assuming a continuous circulation of phosphorus within the plant (ARISZ 1953, BIDDULPH 1941, MASON and MASKELL 1931). It is very unlikely that in the dark the transpiration stream carrying labelled phosphorus from the roots to the leaf is increased. Nor is an increased fixation of phosphorus in the leaf very likely. On the contrary, both processes will rather tend to decrease the phosphorus content if the leaf is kept in the dark. When in spite of this, a gain in phosphorus was observed this must be due to a retarding effect of the dark treatment on the efflux processes.

WEINTRAUB and BROWN (1950) demonstrated that the transport of 2,4-D was connected with the transport of sugars from the leaf to which the growth substance was applied. The same may very well apply to the effect observed on the transport of phosphorus. It is agreed that if sugars would be transported as phosphorylated sugars, as they are often assumed to be, this conclusion would be almost self-evident. However, according to WANNER (1952) carbohydrates in the sieve tubes occur only in their simplest forms owing to the presence of large amounts of phosphatases. Moreover, the preliminary results from experiments, now in progress in this laboratory, show similar effects for labelled rubidium. This suggests the presence of a mass flow from the leaf as assumed in Münch's theory, effected by the sugar production of the leaf.

So far only the influence of light on the leaf in which the activity was measured has been considered. The condition of the other parts of the plant appeared to be of equal importance. Good results could only be obtained if rapidly growing parts were present, which apparently constituted a sink (WILLIAMS 1954). Moreover, changing the light conditions of these parts of the shoots proved to have a profound influence on the labelled phosphorus status of the experimental leaf. Continuing the previous line of thought the lowering of the phosphorus level in this leaf, if the other parts are kept in the dark, may be explained by assuming a lower efflux from these parts, being in the dark, to the experimental leaf. A smaller supply of phosphorus from the other parts is conceivable as the loss of phosphorus from the root tissue to the xylem vessels will depend on the supply from the shoot and the general metabolic activity, both of which are reduced in the dark. In addition there may be an enhanced loss from the leaf as all other parts, in particular the young ones, become dependent on the sugar supply from this leaf.

Thus starting from the idea of a circulation of phosphorus within the plant and the promoting effect of sugar from photosynthesis on the transport through the sieve tubes, a satisfying explanation for the effects observed can be given.

So far experimental data have been discussed as if they represent total phosphorus contents rather then labelled phosphorus contents of the leaf. In fact, alterations in activity can also be explained by assuming changes in specific activity, without any net gain or loss of phosphorus. Therefore the course of the specific activity was studied with a group of bean plants, treated in a way similar to that in the experiments discussed. The results were equal to the expectations. It was found, that at the end of the absorption period specific activity was highest in the roots. During the redistribution period the specific activity of the root phosphorus decreased gradually, while the specific activity of all other parts of the plant increased until after about 5–7 days the specific activity was almost the same for any part of the plant. In particular, no decrease of specific activity in any part of the shoot could be observed. This means that the decreases in activity observed in the experiments described above represent real net losses from the leaf. As a matter of fact, in some cases the net loss may have been a little higher.

On the other hand, the increases in activity, must have partly been due to an increase of the specific activity at least during the first few days of the experiment. However, the results represented by figure 2 show that the changes in specific activity were of minor importance for the greater part of the experiment. Otherwise it would not have been possible to obtain such strikingly similar results in the subsequent light-dark treatments.

Finally it should be realised, that also in case of a mere exchange, i.e. a change in total activity without any change in total phosphorus content, a simultaneous loss and gain of phosphorus must be assumed to occur. Several mechanisms are conceivable but, no doubt, the most rapid one is by way of a supply through the xylem vessels and a loss through the sieve tubes.

SUMMARY

Young bean plants were allowed to absorb a limited amount of labelled phosphorus and were then placed in a minus phosphorus solution. The labelled phosphorus content of one of the primary leaves was studied by a Geiger-Müller tube directly against the leaf. During the first few days of the experiment the labelled phosphorus content increased gradually up to a maximum value. It was followed by a decrease until a constant level was reached, provided the period was long enough. This decrease of the labelled phosphorus content could be stopped and even changed into a increase simply by placing the test leaf in the dark. These effects were obtained irrespective of the light conditions of the other parts of the plant.

However, these conditions did affect the phosphorus status of the leaf. A further loss could be induced by darkening the other parts of the plant. Subsequent illumination of these parts caused the labelled phosphorus content in the experimental leaf to increase again. These results were thought to be consistent with the hypothesis of a continuous circulation of the phosphorus in the plant, involving a steady migration of labelled phosphorus into and out of the leaf, the latter being coupled to the stream of assimilates which is influenced by the light conditions of the leaf.

The question of the role played by changes in specific activity was briefly entered into.

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