RESPONSES OF BEAN PLANTS TO ROOT TEMPERATURES

IV Translocation of ^{22}Na applied to the leaves *

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SUMMARY

The foliar uptake and subsequent transport in bean plants of ^{22}Na, ^{32}P and ^{134}Cs was studied under controlled environmental conditions but at varying root temperatures. The amounts taken up by the plants, held in the treated areas, distributed in various plant fractions or excreted through the roots were determined for periods of up to 6 days. Uptake of ^{22}Na was found to be slower than that of ^{134}Cs and much faster than that of ^{32}P. Root temperature strongly influenced the rate of entrance of the isotopes with an optimum range between 20 and 25 °C. The penetration was found to be more affected by the root temperature during the pretreatment period than by that during the experimental period itself. The translocation and distribution during the first 6 hours of the experimental periods were also influenced by the pretreatment temperature of the roots. Na and Cs leakage in the solution was found to occur at all temperatures, provided transport had allowed their presence in the root tissues.

1. INTRODUCTION

Root environment in general is an obviously important factor in plant growth and consequently in leaf development. Root temperature has been known to play a major role and the topic has been reviewed by RICHARDS *c.s.* (1952). More recently, BROUWER (1962, 1963) has studied the influence of the temperature of the root medium on the growth and dry-matter distribution of seedlings of various crops. Considerable intervarietal differences were reported and later BROUWER (1964), BROUWER & HOOGLAND (1964) and BROUWER & KLEINENDORST (1967) have considered in particular the responses of bean plants to various root temperatures as far as the growth and anatomical aspects were concerned. They found that little growth occurred at low and high temperatures, while a more or less gradual increase in growth to optimal temperatures was followed by a rather abrupt change from optimum to maximum temperature. Leaf size increased with temperature. The number of cells remained more or less constant, variations being due to differences in cell elongation. Leaves were thickest at optimal growth conditions as far as palisade and spongy parenchyma were concerned due to larger intercellular activities but not much difference could be found in either the lower or upper epidermis thicknesses.

* Dedicated to Professor Dr. W. H. Arisz.

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The effects of changes in root temperatures on the general anatomical modifications and metabolic activity of the plant may therefore play an important role in foliar penetration of mineral elements and their subsequent distribution within the plant. The present experiments aimed at determining the influence such changes in metabolic activity and leaf development may have on the initial penetration and subsequent accumulation or translocation of Na, P and Cs applied to the upper surfaces of leaves of young bean plants grown under constant air temperature and humidity. The fate of radioactive Cs contaminant reaching winter crops while the root system is still in colder environment added to the interest of this study.

2. MATERIALS AND METHODS

Bean plants (*Phaseolus vulgaris* var. "Berna") were transferred, after germination in sand at 21 °C, to individually aerated vessels containing a slightly modified Hoagland nutrient solution (1950).

The seedlings were placed immediately, and kept throughout the experiments, at various root temperatures ranging from below to above the optimum for bean plants. In some series of experiments the root temperatures were changed 24 hours or immediately before treatment. Air temperature was kept at 21 °C throughout the 17 hours of light and the 7 hours of dark. Relative humidity was 60–65 % and light intensity at plant height was about 50.000 ergs cm^{-2} sec^{-1} obtained with fluorescent tubes.

Leaf treatment was as described by LEVI (1966 a) and consisted in applying one drop of 0.01 ml aqueous solution containing 0.1 μCi of ^{22}Na or ^{134}Cs in one mM carrier of the corresponding chloride salt or ^{32}P as NaH_2PO_4 to the tip of one primary leaf 3 days after transplanting. At that time, plants at the optimum root temperatures had their primary leaf half developed and the 1st trifoliate leaflet opening. Length of the mid rib varied from 30 mm at 8 °C to 90 mm at 30 °C. At set times after treatment the isotope not retained by the plant was washed off with 10 ml of water and measured. The harvested plant was separated in a number of fractions including the treated area, the rest of the mid rib and the rest of the blades of the treated leaf, each other leaf blade, the stem and the total root system. The nutrient solution was sampled for losses that may have occurred through the roots. The plant material was digested in hot H_2SO_4 and H_2O_2, and aliquots measured in a liquid scintillation counter making use of the Cerenkov radiation effect in the case of ^{32}P, and in a well type NaI(Tl) crystal scintillator in the case of the γ-emitting ^{22}Na and ^{134}Cs.

At least four replicates were used in all cases and the total activity recovered was added to ascertain that no undue losses had occurred. Activity was considered not significant when it was not at least equal to that registered for the background.

Autoradiograms were made on previously freeze dried material to avoid artifacts and following the method described by LEVI (1966 b).

3. RESULTS AND DISCUSSION

The foliar uptake of ^{22}Na, ^{32}P and ^{134}Cs is shown in *fig. 1* for plants grown for three days before treatment and kept thereafter at the root temperatures indicated.

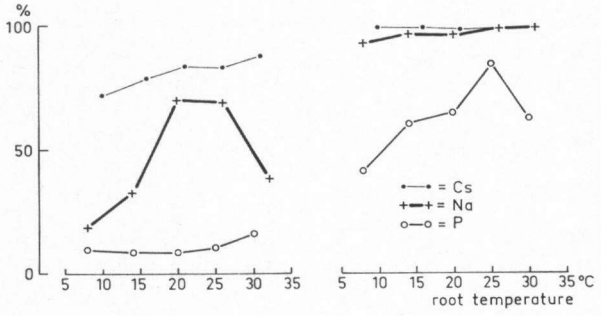

Fig. 1. Percentage of leaf-applied ^{22}Na absorbed by the plants 6 hours (left) and 24 hours (right) after application. The amount absorbed was determined after washing the treated plant part. As comparison the percentages of absorbed ^{134}Cs and ^{32}P in similar experiments are given.

Results of this typical experiment indicate that application of ^{22}Na was followed by a rather rapid entrance into the leaf tissues. Root temperatures strongly affected the rate of entrance with a clear optimum around 20–25 °C; yet, within 24 hours almost all the ^{22}Na had entered the treated leaves in all cases. This Na was retained in a non exchangeable form since it could not be removed by further washings with distilled water or dilute NaCl solution.

Uptake of ^{22}Na was much faster than that of ^{32}P yet slower than that of ^{134}Cs. The reduced speed of entrance of ^{32}P found in the bean variety used in these experiments was still greater than that reported by PHILLIPS & BUKOVAC (1967) in their experiments. It should be pointed out in this respect that considerable variations in uptake were found among various bean varieties (Levi, unpublished results). In foliar uptake experiments under controlled environmental conditions, the rate of uptake varied between experiments without any detectable reason.

The influence of a root-pretreatment temperature on the foliar uptake of ^{22}Na following change of the root environment to the same or other temperatures immediately before application of the isotope is shown in *figs. 2* and *3a*

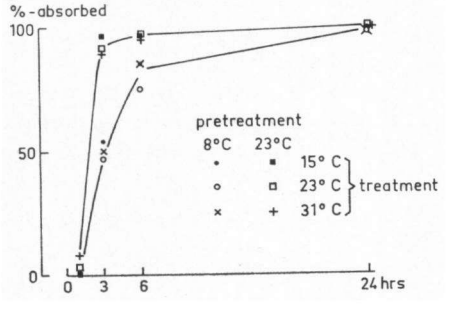

Fig. 2. Differential response of absorption of leaf-applied ^{22}Na to root temperatures during pretreatment and treatment.

for the period 1–24 hours and in *fig. 4* for a period of up to 6 days after treatment. In *figs. 3* and *4* distribution in the plants is given in detail. The data indicate the quantity of isotope not retained by the plant, the quantity held in the treated area, that which had moved in the plant away from that area, and that which had actually moved out of the roots and could be detected in the culture medium kept at temperatures between 8 and 35 °C.

The penetration of ^{22}Na in the bean leaves was consistently more affected by the root temperature during pretreatment than by the root temperature during the experimental period itself indicating the importance of the leaf structure aspect. Besides the differences reported by BROUWER & HOOGLAND (1964) it is conceivable that further alterations to the leaf structure not seen with the naked eye or a normal microscope, such as for instance ectodesmata formation may have played a role in the altered pattern of penetration. This point, however, remains to be confirmed.

At all root temperatures considered, unlike P, and to a lesser extent Cs, sodium was immediately translocated out of the treated area following its absorption.

In the experiments reported here, the isotope was applied 3 hours after illumination had started that day, when carbohydrate transport is supposed to be almost maximal. Transport of these elements in the plants was expected to be influenced by varying the root temperature, since the latter was known to influence carbohydrate translocation. GROBBELAAR (1963) and BROUWER (1964) found that photosynthesis per unit leaf area was in fact independant of root temperature, but translocation of assimilates from the leaves depended largely on root temperature. Roots behaved most efficiently as "sinks" and amounts of carbohydrates transported to the roots were the larger the more optimal conditions in the root medium became. Short term experiments to be reported elsewhere have shown that conditions including low root temperatures which induced a complete inhibition of root elongation do not completely stop carbohydrate translocation to the roots and lead to an accumulation of carbohydrates in the stem base and a high dry matter content of the roots. Although this accumulation is rather pronounced, the rate of translocation in a downward direction is less than in plants with unrestricted root growth, and this is more true, the more unfavourable root conditions are. Recovery following transfer from unfavourable root temperature conditions is not an immediate process, and on the basis of the above mentioned consideration it was expected that both during pretreatment and treatment, root temperatures would influence the rate of carbohydrate translocation but that in none of the treatments would this movement be completely stopped.

Results presented here for ^{22}Na indicate that this element was immediately translocated (*figs. 3* and *4*) following its absorption, the latter in fact being completed rapidly when compared to that of P. Like penetration, translocation was not much affected by the prevailing root temperature but was influenced to some extent by the pretreatment particularly during the first 6 hours following application of the isotope. The distribution of the material transported was even

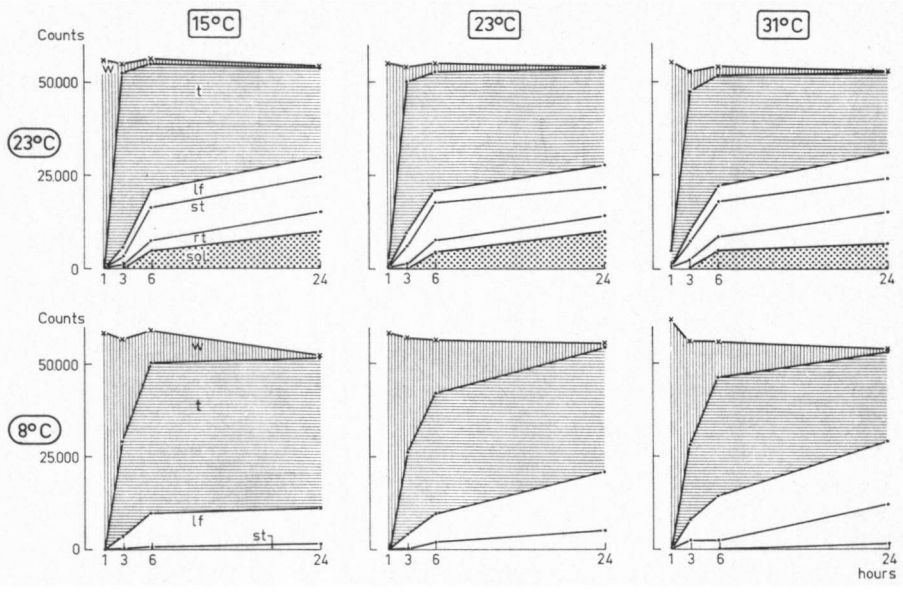

Fig. 3a. Time course of absorption and translocation of leaf applied ^{22}Na in relation to root temperature during pretreatment (circled) and treatment (in squares). Pretreatment period: 4 days, treatment period: 1–24 hours.
The amounts of the various fractions are indicated by: w: washing; t: treated area; lf: all foliage other than treated area; st: stem; rt: roots and sol: loss into the solution.

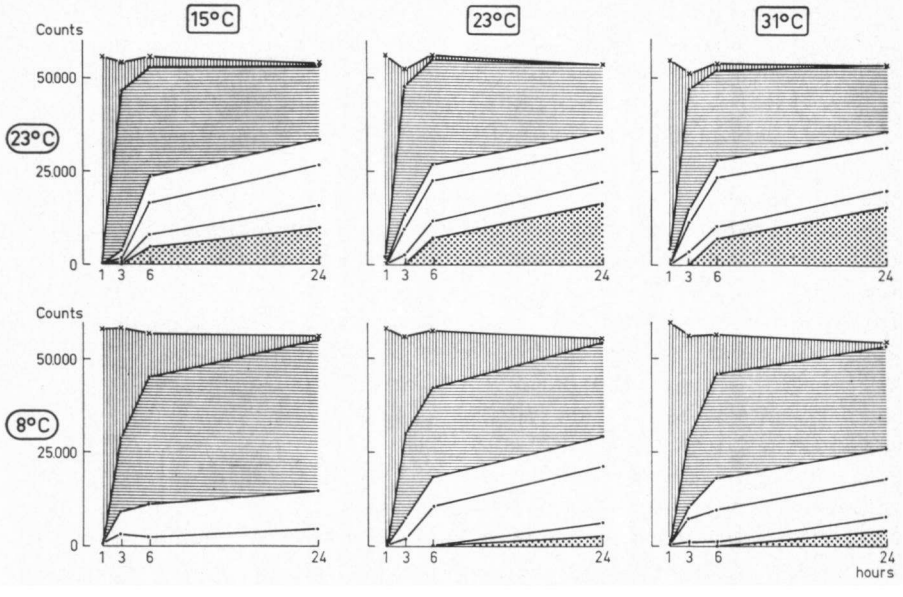

Fig. 3b. As figure 3a but plants transferred to treatment temperatures 24 hours before application of ^{22}Na.

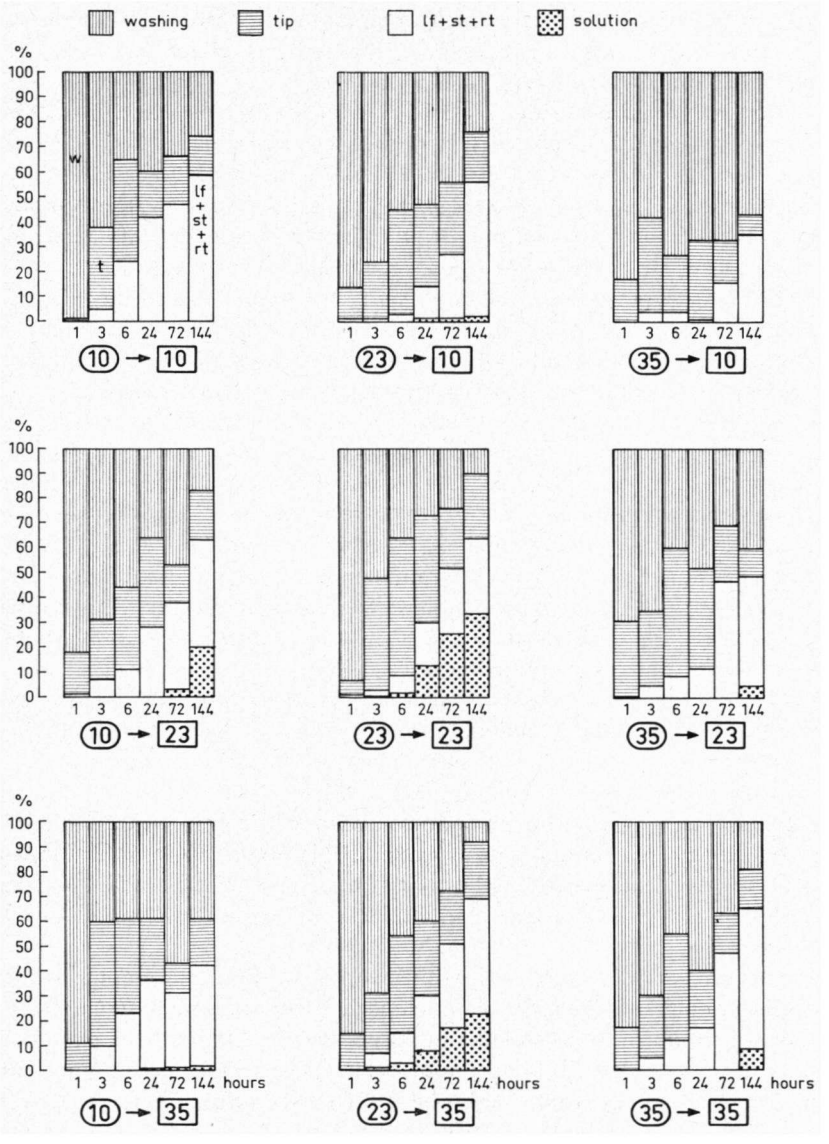

Fig. 4. Diagrammatic representation of absorption, translocation and loss of leaf applied ^{22}Na in relation to root temperatures during pretreatment (circled) and treatment (in squares). Pretreatment: 3 days. Treatment: 1–144 hours.

more influenced by the pretreatment temperatures of the roots. Obviously in the longer period experiments (*fig. 4*), the recovery which had occurred was translated in the transport process.

In the range between 15 and 31 °C, the influence of the prevailing root temperatures on the total transported was negligeable when plants were pregrown at 23 °C. Leakage in the culture solution was slightly higher at the higher root temperatures. In plants pretreated at 8 °C, total transport was much smaller than in the previous group of plants, but the distribution was influenced by the prevailing root temperature. At an experimental root temperature of 15 °C most of the ^{22}Na transported was still confined to the treated leaf in the non-treated area after 24 hours (*figs. 3a* and *b*); at a temperature of 23 °C more of it protruded into the stem and at 31 °C (figs. *3a* and *b*) a small amount of it reached the roots. In none of these cases was a leakage in the solution noted.

Comparing *figs. 3a* and *3b*, the effect of a 24 hour adaptation period to the experimental conditions can be seen. It was small in the plants pretreated at 23 °C root temperature probably due to the rather small differences (maximal 8 °C) between pretreatment and treatment temperatures. For the plants pretreated at 8 °C, the adaptation period to root temperatures of 15, 23 and 31 °C did not affect entrance into the leaves but influenced considerably subsequent translocation. The 24 hour adaptation period resulted in an obvious effect translated by a leakage in the solution at 23 and 31 °C root temperatures, cases where the ^{22}Na had been transported to the roots.

Evidence presented clearly shows that during translocation relatively constant amounts of ^{22}Na could be found in the various parts of the pathway to the roots, even in longer (*fig. 4*) periods of time. This translocation appeared to be only downwards in the case of ^{22}Na. Applied to a leaf, it was never found in significant amounts in parts of the plant above the insertion point of the treated leaf. It is particularly evident that a protrusion of ^{22}Na in the roots was accompanied directly by a leakage whatever the root temperature. The peculiar fact that it moved so rapidly once it had reached the root tissues remains an unsolved problem.

The translocation picture was confirmed by an autoradiographic study. It showed that in plants the roots of which were at near optimum temperature the isotope was confined to the conducting tissue itself (*fig. 5*), indicating that during normal downward transport no important leakage to the surrounding tissues occurred. The isotope could never be detected in autoradiograms of freeze-dried plants at considerable distances from the root base and more often it was just confined to root tissues within 5 cm from the insertion point on the stem, implying a probably very fast leakage through these tissues. The picture differed in plants pretreated at low root temperature. Here, the amounts of Na present were larger and transport downwards was restricted (*figs. 3* and *4*). Autoradiograms show in this case that the isotope was no longer confined so strictly to the conducting tissues and that some leakage from the primary phloem track had occurred followed by a subsequent transport in the xylem to the transpiring mesophyll.

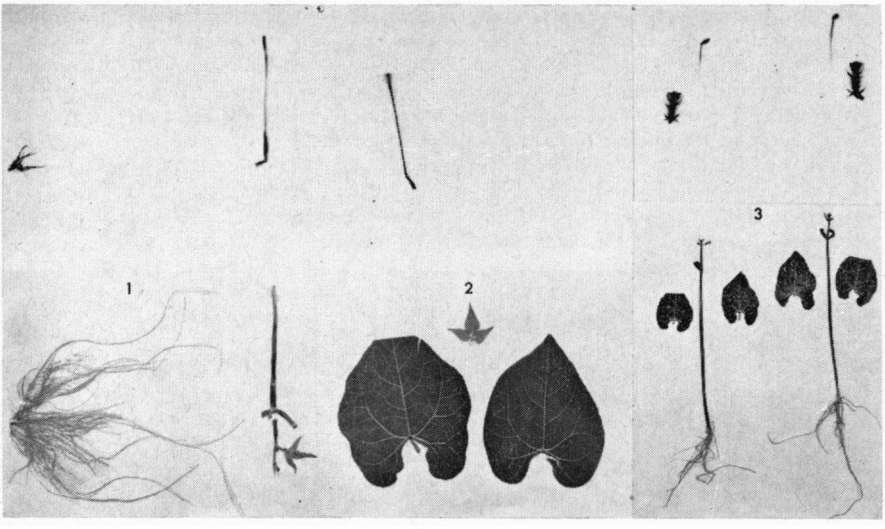

Fig. 5. Autoradiograms of ^{22}Na absorbed by primary leaves of beans (treated tip removed) grown throughout at root temperatures of 21°C (1 and 2) and 8°C (3) 24 hours after application.

The results reported here confirm that the transport of foliarly applied substances follows the carbohydrate flow. In the treated leaves, loading of the phloem is not influenced by the treatments whereas the rate of flow of sugars is. This relation determines the concentration of Na in the flow and how much of the isotope is leaking out and redistributed. Once it has reached the roots, in the cases of Na and Cs, leakage into the solution takes place, particularly rapid for Na, at all root temperatures (details will be published elsewhere). This leads to assume that these losses are not due to active secretion processes.

Results presented indicate the influence root temperature may indirectly have on foliar penetration of inorganic elements due to modifications in the leaf structure. Once absorbed, their transport follows the carbohydrate flow. A rapid leakage of Na occurs in the solution when this element reaches the root tissues.

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