SOME EFFECTS OF LIGHT ON THE MINERAL NUTRITION OF PLANTS

Thesis submitted for the degree of
Doctor of Philosophy

by

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ABSTRACT

Despite the many advances that have been made during the past two decades in the understanding of the mechanism of the absorption of ions by plants, relatively little is still known about their translocation through plants. In several recent publications it has been suggested that the transfer of nutrients across the symplast of the root is a 'passive' process dependent entirely upon transpiration. Two reasons have been advanced in support of this viewpoint. Firstly the close correlation which exists under certain circumstances between the rate of transpiration and the rate at which ions are transferred to the plant shoot. Secondly the evidence from many sources that the outer surface of the cytoplasm does not present a high resistance barrier to the entry of ions - that is to say the concept of apparent free space.

As against this concept of passive movement there has existed for the past two decades a considerable body of evidence which has been regarded as establishing the dependence of nutrient accumulation on metabolism. The widely known

work of Hoagland and Broyer is particularly relevant. These workers showed that whereas variations in the rate of transpiration exerted a large effect on the movement of ions to the shoots of plants of initial high nutrient status, only a small effect was observed when the initial nutrient status of the plants was low.

The recent suggestion of Hylmo and Epstein that ions are transferred passively across roots rests on Hylmo's observation that transpiration and the translocation of ions to the shoots of young pea seedlings varied concomitantly. Since the seedlings were of high salt status his results accord with those of Hoagland and Broyer. The tacit conclusion that the relationship observed by Hylmo between transpiration and translocation is of general occurrence however appears unwarranted without further consideration of the effect of transpiration on uptake by plants of different salt status and by plants supplied with different concentrations of salts.

Accordingly an investigation of this subject was undertaken. Transpiration was varied by shading and the duration of the experiments was sufficiently short for the effects of shading other than that of

varying the rate of transpiration to be negligible. Phosphate and rubidium, labelled with ^{32}P and ^{86}Rb, were the ions studied. Barley was the principal experimental plant, but in some experiments sunflower plants were used. The work was carried out in water culture. The plants were raised in a greenhouse, and when they had attained a suitable size and had been subjected to any necessary pretreatment to adjust their nutrient status, they were sealed in the experimental vessels which contained the appropriate radioactive nutrient solution. They were then transferred to the controlled environment chambers where they could be exposed in the dark or in varied light intensities, under constant conditions of temperature and humidity. Transpiration losses were determined by weighing, and the absorption of nutrients was determined by the assay of roots and shoots separately.

The extent to which the rate of transpiration affected the transfer of phosphorus from the root to the shoot varied both with the concentration of phosphorus supplied to the plant and the initial phosphate status of the plants. When low levels of phosphorus (0.1 p.p.m. P) were employed and the initial phosphate status of the plants was low, the

shoot content was either independent of transpiration or was only slightly affected by it. When however the concentration of phosphorus in the external medium, or the phosphate status of the plants was increased, a close correlation between shoot content and transpiration occurred. With rubidium also transpiration affected the shoot content more when rubidium supplied was supplied at a high concentration (85 p.p.m. Rb), but some effect of transpiration remained at the lowest concentration employed (0.28 p.p.m. Rb). Qualitatively therefore there is a similarity between the effects of transpiration on the absorption of both phosphorus and rubidium.

These findings, which are in close agreement with those of Broyer and Hoagland, are most simply interpreted as indicating that ions are not transferred from the external medium across the root and thence to the shoot by the transpiration stream, but that one step in the overall process, which can on occasions be rate limiting, is dependent on transpiration. This situation occurs when the external concentration of ions is high, and especially when the plants are of high nutrient status.

Evidence of many types shows that the transpiration dependent step is not the transfer of

ions from the external medium across the symplast to the xylem: the correlation between oxygen uptake of plants and salt uptake is well established: respiratory inhibitors have been shown to reduce both the absorption and the translocation of ions to the shoots, in a manner similar to the findings in tissue slices. The present investigation provided further evidence on this subject.

Since phosphate and rubidium in the transpiration stream are present predominately as free ions, it is legitimate to calculate the mean concentration of ions in the transpiration stream, from the quantity of the ions in question which reaches the shoot and the quantity of water transpired. When transpiration is low and the concentration of salt supplied to the plant is small, the mean concentration of ions in the transpiration stream may exceed that in the external medium by factors greater than 100. Moreover when the external concentration is high the T.S.C.F. may be significantly less than unity. The high concentration gradient against which ions are moved is conclusive evidence of the expenditure of metabolic energy in the process of translocation to the shoot. Furthermore the existence of significant parallel pathways for passive diffusion is precluded

since their existence would preclude the possibility of the large concentration gradients being maintained between the internal and external concentration.

It is considered that the interpretation suggested by Hoagland and Broyer is supported by the present evidence, namely that transpiration can affect the rate of upward movement to the shoot of ions which have already been released into the xylem. This step may become rate limiting in the overall process of translocation when the external concentration of salt is high and/or the plants are low salt status.

The high internal concentration of ions suggests the existence of a barrier against the free diffusion of ion into the xylem. Its effect may be compared to that of the tonoplast of a single cell. Anatomical considerations suggest that the endodermis may be the site of this barrier. This in no way disputes the possibility that considerable zones of the root cortex are not separated from the external medium by a high resistance barrier.

Some observations were made on the effects of shading individual leaves on their accumulation of phosphate and rubidium. Sunflower plants were used for this work. During the short experimental periods employed, shading of an individual leaf

reduced the content of that leaf. As it would be expected that shading would reduce the rate of transpiration from a leaf, the effects observed accord with the postulate that the transpiration stream passively transports ions in the xylem.

Having observed the large effect that transpiration exerted on the absorption and translocation of phosphorus and rubidium, consideration was given to the effects of transpiration on salt uptake by plants that had been grown continuously under three levels of light intensity, ranging from full daylight to 14% full daylight. The experiments, which were of a preliminary nature, were carried out in the open. Immediately prior to the 24 hour treatment period in which the absorption of phosphorus and rubidium was measured, the plants from each light intensity were divided into three groups and were then treated under the three levels of light.

In contrast to the experiments carried out in the controlled environment chambers, variations in the transpiration rate had but a small effect on salt uptake. It is postulated that this situation was due to the fact that in the plants which had been grown and treated under the same level of light intensity and with the same concentration of nutrients,

that a 'balance' had been attained between the metabolic processes responsible for the active transport of ions across the root, and the utilisation in the shoot, with the result that variations in the rate of transpiration could exert but a small effect on the upward movement of ions.

A markedly different situation occurred in the experiments carried out in the controlled environment chambers since all plants were subjected to a marked environmental change during the treatment period. It appears that all reported observations of the effect of transpiration on salt uptake have been obtained under artificial conditions. It is apparent that the striking effects of transpiration on upward movement of salts only occurs under such conditions. Some modification of the distribution of nutrients within the individual leaves may nonetheless result from natural shading.

Thus the present data suggest that the effect of constant shading on the nutrient balance of plants is to be explained in terms of the effect of variations in light intensity on metabolic processes.

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I. INTRODUCTION

The study of nutrient absorption and the immediate distribution of ions which enter intact plants can conveniently be divided into two aspects, firstly the entry of ions into the root and secondly the transport of ions to other parts of the root and the shoot. By far the greatest amount of information concerning the absorption of ions has been gained by investigations using algae, tissue slices, and excised roots as the experimental material. The use of such simple tissues facilitates the examination of the initial processes of absorption but precludes the study of translocation. The absorption of ions by intact plants has been studied less frequently, (Steward, Prevot and Harrison 1942, Broyer and Hoagland 1943, Alberda 1948, Woodford and Gregory 1948, Broyer 1950, Helder 1952, Butler 1953a, Russell and Martin 1953, Russell, Martin and Bishop 1953, 1954). However, despite the many advances that have been made concerning the initial absorption of ions, the factors which affect the transport of nutrients in intact

plants remain largely obscure. In an endeavour to provide further information on this subject, it seemed desirable to investigate into the effects of light on the absorption and transport of ions in intact plants. It was realised that the effects of variationsin light intensity on plant growth are of great importance in plant competition studies.

The most conspicuous role of light in mineral metabolism is through photosynthesis. A carbohydrate supply is necessary as a source of energy for the accumulation of ions by roots (Hoagland and Broyer 1936, Steward 1935), and for the carrying out of all other chemical transformations driven energetically by respiratory reactions. The mineral elements enter into synthesis with substrates derived from the products of photosynthesis to form the metabolic and structural components of plants. It therefore follows that the mineral requirements of plants is directly dependent on the carbohydrate supply and in turn upon photosynthesis. The absorption of sunlight by leaves and other portions of the shoot, and by the soil surface frequently results in the temperature of the shoot, and to a lesser extent the root being significantly higher then the surrounding air temperatures. This

rise in temperature due to the absorption of radiant energy may become sufficiently high to accelerate thermal reactions, but more important, to alter markedly the transpiration rate. Evidence to be discussed later suggests that the distribution of ions may be greatly affected by the rate of transpiration. The effects of variations in light intensity on growth have been extensively studied by Blackman and Rutter (1948), Blackman and Wilson (1951a, 1951b, 1954), and Hodgson and Blackman (1956). In particular it is to be noted that with an increase in light intensity there occurs both an increase in the weight of root per plant and also in the root/shoot ratio. Thus as the light intensity is decreased the balance of metabolic activity within the plant is swung towards a relatively greater activity of the shoot. On the basis of the postulate that metabolically unattached ions move towards points of utilisation or 'sinks' (Williams, 1955), it would be expected that the distribution of ions would be affected as the pattern of metabolic activity changes. Changes in the light intensity may also alter the formation, inactivation or mode of action of growth regulating substances in

plants (Blackman and Robertson-Cuninghame 1955a, 1955b, Weintraub and Brown 1950, Avery et al 1937, Went 1951).

Thus light may influence the mineral nutrition of plants in three distinct ways :-

(i) By affecting the entry of ions into the root and the transference to the vascular stele, brought about by changes in the carbohydrate supply to the roots, and by the magnitude of the metabolic retention of ions in the root.

(ii) By affecting the utilisation of ions within the plant brought about by changes in the growth pattern.

(iii) By affecting the initial movement of ions from the roots, possibly by an effect of the rate of water movement across the root and also in the vascular tissue.

Whereas a great deal of information is available concerning the processes involved in the initial entry of ions to the root, and to a lesser extent indirect information on the effect of altered patterns of growth on the tuilisation of minerals, the effect of

the rate water movement, as controlled by the light intensity, on the absorption and distribution of ions is a subject on which there exists a distinct diversity of opinions. Accordingly in the present study attention was directed to the effects on the initial distribution of ions within plants, which result from the changes in the rate of transpiration, which are induced by variations in the light intensity. The phosphate and rubidium ions were chosen for study. The use of tracer techniques made it possible to employ short treatment periods of 24 hours. In this way it was found possible to eliminate the effects of a reduction in the light intensity on the absorption of phosphorus and rubidium, other than those brought about by changes in the rate of transpiration.

The relationship between transpiration and the absorption of nutrients was considered by Sachs (1887), Pfeffer (1900) and Jost (1907), who were of the opinion that in intact plants the transpiration stream could possibly transport salts from the external medium into the plant by a purely passive process. No connection between salt uptake and the rate of transpiration, varied by shading, was by contrast observed by

Hasselbring (1914), Kiesselbach (1916), Mendiola (1922) and Gracenin (1932). Their results cannot be regarded as conclusive as the experiments lasted for long periods of time (60 days in the investigation by Hasselbring), during which time marked physiological and morphological differences must have developed between the plants in the different treatments. Moreover, the mineral nutrition of the plants was not rigidly controlled. Other investigators have demonstrated a definite connection between water transport through the plant (varied by shading or by different levels of humidity) and ion uptake, (Freeland 1936, 1937, Wright 1939, Schmidt 1936, Bottcher and Behling 1939, Phillis and Mason 1940, Michael and Wihlberg 1951).

However, several more recent investigations have shown conclusively that the rate of salt uptake is little affected by the rate of transpiration. In this respect the work of Hoagland (1943), Broyer and Hoagland (1943), and Hoagland (1948), is of particular interest. It was shown that salts can be absorbed from the external solution either more or less rapidly than water, depending upon the type of salt, the salt status of the plant and its sugar content. Broyer

and Hoagland (1943) describe an experiment with 19-day old barley plants of low salt-high sugar status. In a 12-hour treatment period no significant difference in the amounts of potassium or bromide absorbed was detected between the plants treated in the light and dark, despite the large differences in the amounts of water transpired. The rate of transpiration had however a slight effect in that the increase in the salt content in the upper part of the shoot was greater in the plants transpiring at the high rate. In other experiments Broyer and Hoagland showed that when plants of high salt status were treated in the light and dark, a different picture was observed, namely that the higher the rate of transpiration the greater the amounts of potassium and bromide absorbed. Broyer and Hoagland concluded that "the data presented on young barley plants oppose the idea that either the absorption or the translocation of salts necessarily depends on the absorption of water or on transpiration, even though it may be possible to arrange conditions so that more salt is absorbed when more water is absorbed". Broyer and Hoagland also mention a striking example of the effect of the rate of transpiration on the translocation of radioactive bromide,

which could be detected in the tops of rapidly transpiring cotton and cucumber plants within ½-1 hour of being supplied to the plants, but not in weakly transpiring plants.

A considerable body of evidence based on the effects of temperature, oxygen supply and the effects of inhibitors indicates that the absorption of ions is dependent upon metabolism, (e.g. Hoagland and Broyer, 1936, 1942, Vlamis and Davis 1944, Ulrich 1941, Steward 1933, 1935, 1947), and the investigations so far reviewed thus point to the conclusion that the effects of transpiration are of secondary importance. Hoagland (1948) concluded that the rapid removal of salt from the roots makes conditions favourable for the continued active movement of salt into the xylem. Lundegardh (1943, 1945, 1950), Phillis and Mason (1940) and Freeland (1936, 1937) also advanced similar opinions.

Recently there has been a resurgence of interest in this subject. Hylmo (1953) observed a close correlation between the absorption of water and the absorption of calcium and chloride by 20 day-old intact pea seedlings. This observation accords with the results of Broyer and Hoagland since his pea seedlings

were supplied with calcium and chloride before the treatment period and were hence of high salt status, also he used relatively high concentrations of $CaCl_2$(1-11 m M). Hylmo has however claimed that his results indicate that ions are transported from the external medium across the root to the xylem passively in the water stream; this interpretation is in direct conflict with that of Hoagland and Broyer.

More recently Brouwer (1953, 1954, 1956), has found that the rate of transpiration can markedly affect the uptake of chloride, nitrate and phosphate by maize and beans.

By contrast, the work of van den Honert (1955) on the absorption of ammonium, nitrate, potassium and phosphate ions by maize plants submitted to conditions of low and high transpiration showed no effect of transpiration on ion uptake. Since Brouwer used a Hoagland solution with a higher phosphate concentration (44 p.p.m. P) and a higher nitrate concentration (158 p.p.m. N) than used by van den Honert (5-12 p.p.m. PO_4 and 42-48 p.p.m. NO_3), it appears that the difference between the results could be due solely to the different concentrations of salts used. This conclusion is supported by the fact that Schmidt (1936)

who worked with the same experimental plant (Sanchezia nobilis) to van den Honert (1955) but employing concentrations of phosphate and nitrate 50-100 times than those used by van den Honert, found a strong correlation between absorption and transpiration, whereas van den Honert observed only a small effect of transpiration on absorption. Despite the work of Hoagland and his colleagues, van den Honert and Brouwer do not seriously consider the fact that the effect of transpiration on absorption may vary according to the concentration of salts supplied to the plants.

Further data relevant to this problem are provided by Alberda (1948) and Helder (1952), who however were primarily interested in the relationship between growth and ion uptake in maize plants. In experiments where plants were moved from the light to the dark, Alberda found that the uptake of phosphorus rapidly came to a standstill only when the plants were of a high salt status. When the plants were of low salt status uptake of phosphorus continued for at least 48 hours in the dark. Also Helder with similar plant material and treatments observed that with plants of low salt status, the rate of uptake of phosphorus, supplied at a high level ($\doteq$ 7 p.p.m. P),

rapidly declined when the plants were placed in the dark, whereas when a low level of phosphorus ($\simeq$ 2 p.p.m. P) was supplied, only a slight reduction in the rate of uptake was observed. Neither investigators considered the possible role of transpiration on ion uptake, and they interpreted their results in terms of growth and the carbohydrate level of the roots.

The contrasting conclusions of Hylmo, Brouwer and van den Honert have turned the subject, which at an early stage seemed to have been settled, into a matter of controversy. Epstein (1956a), (1956b) has recently favoured Hylmo's viewpoint, since it apparently fits with his view of the diffusion of ions in the cytoplasm. Hylmo's original paper, which initiated this controversy, appeared shortly before the present investigations were commenced, and it was considered to provide an additional reason why the study of the effect of transpiration on salt uptake was a logical first step in an investigation of the effects of variation in light intensity on nutrient absorption. The initial experiments were of short duration so that the effects of light intensity other than that of varying the rate of transpiration could be reduced to a minimum. Subsequently less

detailed investigations on the effect of shading individual leaves, and on the effect of shading of whole plants for considerable periods were carried out. (Part II).

II. EXPERIMENTAL METHODS

The plants were raised in the main similar to the method employed by Russell and Martin (1953): barley was the principal experimental plant but in some experiments sunflower plants were used. The plants were raised in a greenhouse where only a limited control of the environment was possible. When the plants had attained a suitable size in the greenhouse, and had been subjected to any necessary pretreatment to adjust their nutrient status, they were sealed in the experimental vessels which contained the appropriate radioactive nutrient solution. They were then transferred to the controlled environment chambers in which they could be exposed in the dark or in varied light intensities, under constant conditions of temperature and humidity. Transpiration losses were determined by weighing, and the absorption of nutrients was determined by the assay of roots and shoots separately. Details of the procedures are described below.

1. Culture of Barley Plants.

The investigations were carried out in water culture, this enabled the mineral nutrition of the plants

to be controlled, and also provided a simple method of supplying the radioactive tracers (^{32}P and ^{86}Rb). The work of Russell and Martin (1953) has shown that barley was a suitable experimental plant for this type of investigation and one that presented no culture difficulties. Pure lines are readily obtainable and the variety employed was 'Proctor', provided by the National Institute of Agricultural Botany, Cambridge.

Experiments were carried out at all times of year and all plants were cultured in a greenhouse, where a limited control of the environment was possible. Thermostatically controlled heating prevented the temperature from falling below 10°C in the winter, and in the summer movable blinds, 'summer cloud' and thermostatically operated fans were employed to prevent excessive increases in temperature. From September to March, fluorescent lights were used to prevent the photoperiod from falling below twelve hours.

The seedcoats were sterilised by immersing the grain in a 0.1 per cent mercuric chloride solution for thirty seconds, followed by a thorough washing in demineralised water. The seed was germinated in the dark on trays lined with filter paper moistened with demineralised water. When the seminal roots were

approximately one centimetre long the most uniform seedlings were selected and transferred to a black polythene tank (40 cm. x 45 cm. x 21 cm.)(Plate 1), containing the appropriate nutrient solution (Table 1) diluted to 1/5 strength. Previous work (Martin 1951) had shown that the seedlings could conveniently be supported in the tanks between pairs of internally blackened glass tubes. The space between the tubes was wide enough to allow free passage of the roots, but was narrow enough to prevent the seed from falling through. Aeration and circulation of the nutrient solutions in the tanks was provided by means of 'aloxite' blocks on the ends of glass tubes connected to an air supply line. When the second leaf was developing the plants were graded for size and placed temporarily in culture vessels containing 300 ml. of nutrient solution (pH 6.0 ± 0.2).

The plants were supported by cotton wool plugs in slotted waxed corks (Plate 2), which fitted tightly into the neck of the vessels. An aerator device slightly modified from that described by Martin (1951) which was connected to the air supply line, was used for aeration and circulation in the vessels (Plate 2). The vessels were wrapped in black cloths, and in hot weather the trays on which the vessels stood were filled

TABLE 1.

Composition of Culture Solutions.

A. Major Nutrients.

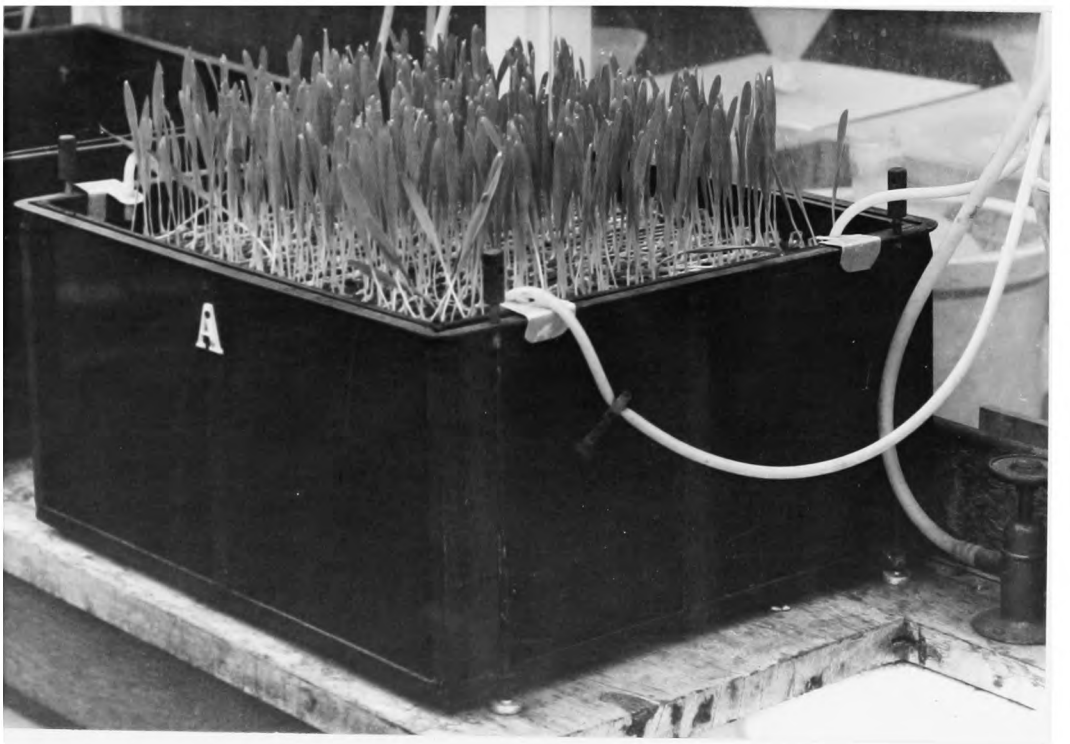
	complete culture solution		phosphate-free solution		potassium-free solution	
	m.e/litre	p.p.m.	m.e/litre	p.p.m.	m.e/litre	p.p.m.
Mg^{++}	3	36	3	36	3	36
Ca^{++}	3	60	3	60	9	180
K^{+}	6	225	6	225	-	-
Na^{+}	2	46	2	46	2	46
NO_3^{-}	10	620	10	620	10	620
SO_4^{--}	3	144	4	192	3	144
$H_2PO_4^{-}$	1	97	-	-	1	97

The above solutions were prepared from

$MgSO_4$ (A.R.); $CaNO_3$; KNO_3(A.R.);
K_2SO_4 (A.R.); $NaNO_3$ (A.R.)

B. Minor Nutrients.

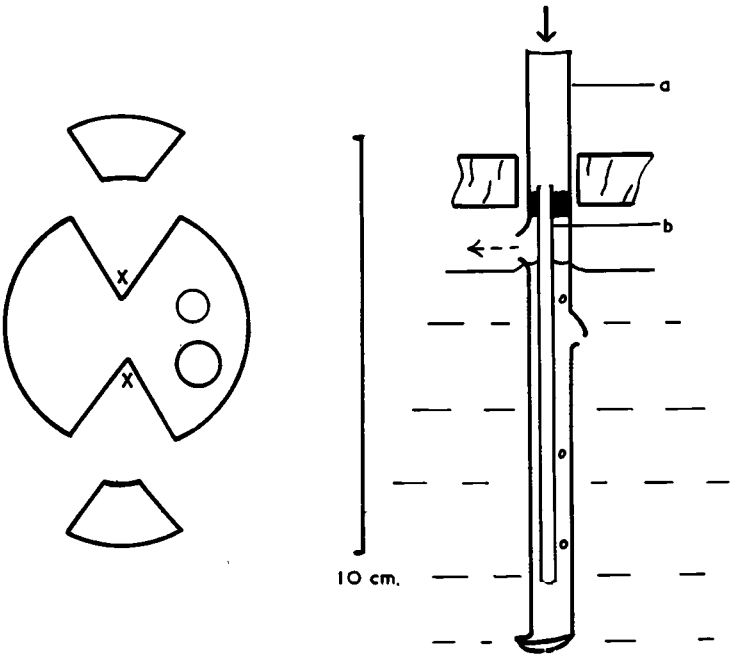
	'single' strength	'double' strength	'quadruple' strength
		p.p.m.	
Fe^{+++}(Ferric Citrate)	0.30	0.60	1.2
Mn^{++}($MnSO_4$ $4H_2O$ A.R.)	0.25	0.50	1.0
B^{+++}($Na_2B_4O_7$ A.R.)	0.28	0.56	0.56



Tank used for raising barley seedlings.

The plants are ready for transfer to the culture vessels.

Plate 2.



Left: Diagram of cork cut to support seedlings in the culture vessels.

X indicates the position of seedlings.

Right: Diagram of aerator devise.

The arrows indicate the direction of air flow.

with water; the evaporation of water from the cloth prevented excessive increases in the temperature of the nutrient solution.

The plants, usually four per vessel, remained in the 300 ml. vessels, which were employed before transference to the transpiration vessels, for a period of one to five days, depending on their rate of growth and on the nature of the pretreatment. Plants possessing two expanded leaves were normally used. In some experiments where low levels of phoshorus and rubidium were used, larger culture vessels were employed so that depletion of the nutrient solution, with regard to the tracer used, might be minimised. In these cases, 1500 ml. polythene tanks holding four plants were used, and the slotted waxed corks holding the plants were supported by aluminium covers.

2. Culture of Sunflower Plants.

The variety of sunflower used was a dwarf variety 'Pole Star' which is suited to the conditions of Great Britain (Blackman 1946). Much information has been gained using this variety by Blackman and Wilson (1951a), (1951b), (1954) and Blackman, Black and Kemp (1955) concerning the relationship between light intensity and growth.

Selection of the seed was carried out prior to germination in order to eliminate cracked, small and suspected diseased seeds. The seeds were germinated in the dark in trays lined with filter paper, moistened with demineralised water. As the experiments were carried out in water culture not only was it necessary to germinate the seed before transference to the culture vessels, but it was also necessary to allow the seedlings to grow to a stage when they could be conveniently handled. This was done by raising the seedlings in vermiculite; the germinated seeds were planted in the vermiculite when the radicle was 0.5 - 1.0 cm. long. When the hypocotyl was long and robust enough to be held in a cork the seedlings were removed from the vermiculite by washing the vermiculite from the roots, and transferred to the culture vessels. Unless otherwise stated a complete culture solution, with a 'double strength' minor nutrient solution was used in the culture of sunflower plants.

3. Vessels used for the measurement of transpiration and nutrient absorption.

The plants, sealed in circular black polythene caps, were fitted over the 250 ml. beakers which contained the appropriate labelled solution (Plate 3). The level

Plate 3.



Vessel used for the measurement of transpiration and nutrient absorption.

For explanation see text.

(p. 17)

of activity used was 5μC per litre. The plants were secured in the holes in the caps by the use of small slotted corks and plugs of cotton wool covered with resin cerate. The caps, each with a bevelled groove on the underside, fitted snugly onto the lip of the beaker, which was also covered with resin cerate as a further protection against the spurious loss of water. At the commencement of the treatment period during which the absorption of the appropriate nutrient was measured, the caps were fastened onto the beakers by a spring clip (Sutcliffe preserving jar clip), and the sealed plants were then exposed to the appropriate light intensity. It was observed that in the absence of plants no significant water loss occurred through the seal (< 0.1 g. in 24 hours).

4. Controlled Environment Chambers.

Two controlled environment chambers maintained at 20 ± 2°C were used (Plate 4): the one illuminated(A) from above by twelve 5', 80 watt fluorescent Osram daylight tubes, situated 22" from plant base level, and separated from the chamber by a perspex sheet, giving a light intensity of 500 f.c. at plant base level: the other was not illuminated(B). For convenience the chambers were mounted the one above the other. In some experiments

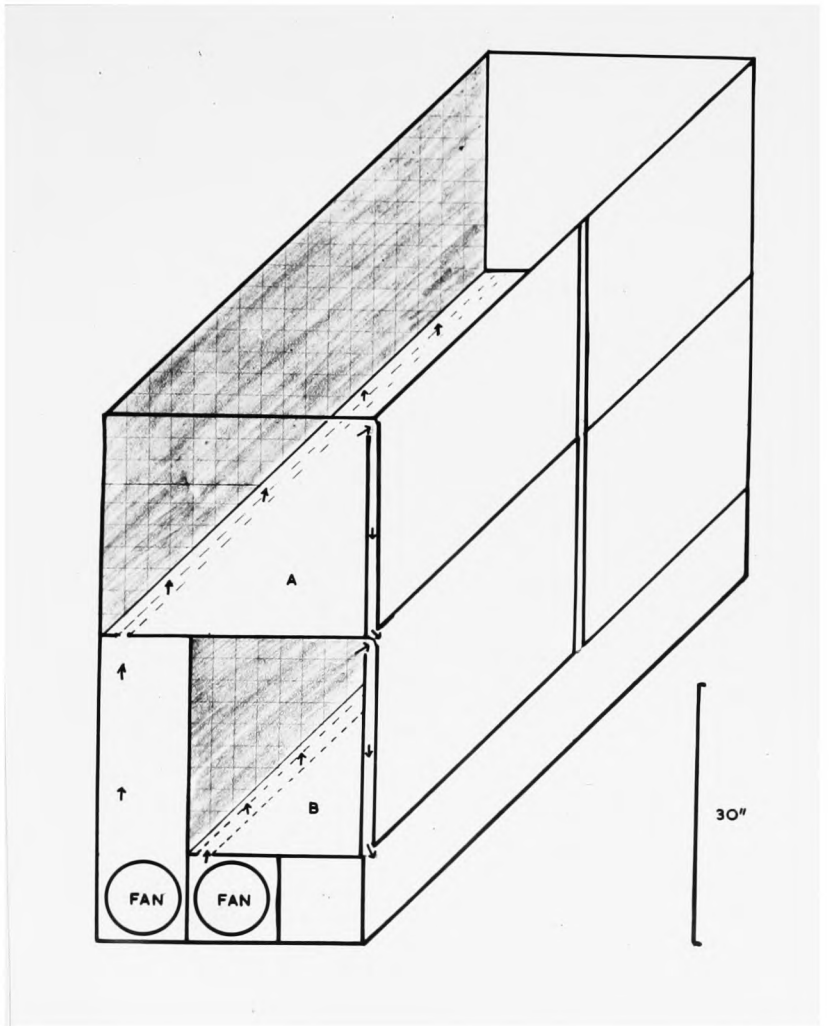


Diagram of the Controlled Environment Chambers.

The arrows indicate the direction of airflow.
For explanation see text.

TABLE 7.

Experiment 10. The effect of shading on transpiration, the plant dry weights and the absorption and distribution of phosphorus by barley plants pretreated for 48 hours with two levels of phosphate and a control, and treated for 24 hours in solutions containing 31 p.p.m. and 93 p.p.m. P.

Pretreatment Concn. P	0				31 p.p.m.				93 p.p.m.				S.D. (P= 0.05)
Treatment Concn.P	31 p.p.m.		93 p.p.m.		31 p.p.m.		93 p.p.m.		31 p.p.m.		93 p.p.m.		
	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	
Transpiration-g. water	4.3	0.8	4.4	0.8	4.8	0.8	5.3	0.9	5.3	1.0	5.5	0.9	0.1
" -g/g dry wt.shoot	36	8	37	8	38	7	41	8	40	9	41	09	2.1
Dry weight mg. roots	35.7	33.7	36.5	32.2	33.0	31.2	35.0	32.2	35.2	31.7	35.5	31.0	4.5
shoots	119.2	107.2	119.2	108.2	124.0	109.5	130.0	112.0	134.0	107.2	133.7	106.7	10.1
Phosphorus contents µg.													
root	107	69	238	211	82	62	233	205	92	52	222	162	25
shoot	356	152	373	159	257	92	284	90	269	74	310	111	50
total plant	463	221	611	370	339	154	517	295	361	126	533	273	68.5
Conen. phosphorus in transpiration stream p.p.m.	82.3	184.1	83.7	187.7	53.8	111.5	53.3	101.9	50.8	76.1	56.7	124.2	21.7
T.S.C.F.	2.6	5.9	0.9	2.0	1.7	3.6	0.6	1.1	1.6	2.4	0.6	1.3	
	1.42	1.77	0.94	1.30	1.23	1.55	0.76	1.04	1.21	1.38	0.76	1.13	0.15
Transport Index %	77	68	61	43	76	59	55	31	74	59	58	41	4.1

ion

Where a logarithmic transformation was necessary for statistical analysis the transformed values are underlined.

plants during the short treatment periods were negligible when compared with the weight of water transpired. The vessels were weighed at the outset, during and at the end of the treatment period to an accuracy of ± 0.05 g.

It may be noted that during the short treatment periods the plants did not absorb significantly less phosphorus than plants in aerated culture solutions. The pH of the unaerated treatment solutions was 5.7 ± 0.2.

6. Shading of individual leaves.

In a few experiments which are qualitative rather than quantitative, individual leaves only were shaded. Sunflower plants were used in this work. The plants were raised in the manner already described, and the experiments were carried out in the greenhouse.

Shading was effected by enclosing individual leaves in sleeves of butter muslin. The light intensity under the butter muslin was reduced to approximately 0.5 daylight. The muslin sleeves, of triangular cross section, were held rigid by a wire frame: the whole shade could then be conveniently held by a clamp. The leaf was enclosed without the muslin touching the leaf. The open ends of the shade were covered with butter muslin after the shade had been fitted.

7. Tracer Procedures.

(a) Plant Sampling.

At the end of the treatment period the plants were removed from the polythene caps. The seed coat (barley) was removed and the roots, cut off at the point of junction with the shoot, were quickly washed in demineralised water to remove any adhering nutrient solution. Two plant samples were taken, namely root and shoot.

The plant samples were placed in dishes and dried in an oven for twentyfour hours at 100°C. After cooling in a dessicator, dry weightsof the samples were recorded. 10 ml. samples of the treatment solution were also taken, so that a 'balance sheet' of the activity in the roots, shoots and solution could be calculated, and compared with the control solutions in which plants were not grown, thus providing a check on the results. The 10 ml. aliquots of the labelled phosphate solutions were acidified by a few drops of phosphoric acid to prevent loss of phosphate by adsorption onto the specimen tubes and also to prevent the possible contamination of the counter tube.

(b) Preparation of Samples for Assay.

Wet ashing of the dried plant samples was employed throughout the investigation, to destroy all the organic matter, and to bring into solution all the inorganic constituents. By this method liquid counting of the samples could be carried out. Previous work by Martin and Russell (1950) showed that dry ashing of samples led to losses during combustion and to the contamination of the furnace, whilst wet ashing resulted in insignificant losses, and was readily adaptable to routine work.

The plant material was placed in 50 ml. or 100 ml. Kjeldahl flasks, depending on weight of sample and a quantity of nitric acid added, again depending on weight of sample, followed by 2 ml. of concentrated sulphuric acid. The Kjeldahl flasks were heated on an electric hot plate in a fume cupboard until all the nitric acid was removed and a colourless or straw coloured liquid remained, which became colourless on cooling. Further nitric acid was added and the flasks reheated if ashing was not complete. The time taken by the process varied from half an hour (for very small samples) to several hours.

Subsequent to ashing the samples were diluted with demineralised water and made up to 10 ml.

and stored in specimen tubes.

(c) Estimation of ^{32}P and ^{86}Rb.

In the majority of experiments the liquid counting techniques described by Martin and Russell (1950) using type M.6. Geiger-Muller counter tubes was employed to measure radioactivity. The liquid sample (10 ml.) was placed in the tube, of this only 9 ml. is sufficiently close to the thin walled part of the tube to contribute to the recorded counts (Veall, 1948). Thus small variations in the volume of solution decanted into the tube do not cause errors, and any drops of liquid remaining in the specimen tube may be ignored. The counter tube was enclosed in a 'lead castle' during assay, which reduced the background count to 10 - 12 counts per minute. The castle also excluded light, an important factor with M.6. counter tubes, some of which are photosensitive.

After the daily routine check on the efficiency of the counter tubes by counting a uranium standard, the background count was determined using demineralised water. If this was satisfactory the counter tube was dried using acetone and the sample for assay introduced. The counting rate was then determined over an appropriate period depending on the activity of the sample. The decay of all radioactive

substances is characterised by a random distribution of disintegrations in time, thus the Poisson distribution is applicable, and the standard error of an observation of the disintegration rate is equal to the square root of the number of counts observed, Fisher (1948). Thus to obtain a standard error of 1% it is necessary to extend the counting time over a period in which at least 10,000 disintegrations are detected. The need to assay samples fairly rapidly made prohibitive the use of counting times longer than 20 minutes.

Between samples the counter tube was washed three times in demineralised water and dried with acetone.

8. Calculation of results.

The following factors were taken into account when calculating the activities in millimicrocuries from the observed counting rates.

(a) Unrecorded counts.

The resolving time (the minimum time interval between events which a system is capable of distinguishing) of the quenching unit is three hundred microseconds, hence the counter is insensitive to any pulses received from the counter tube

during this period. The number of unrecorded counts is thus dependent on the counting rate of the sample, the higher the counting rate, the greater the number of unrecorded counts. Tables showing the number of unrecorded counts at any counting rate were used to correct the observed counting rate figure.

(b) Background count.

This was determined daily and was subtracted from the observed counting rate.

Using the above two corrections the true counting rates were deduced and then multiplied by further factors to derive the activities of the samples.

(c) Radioactive decay.

To allow for the reduction in activity of the sample due to decay during the period between the experiment and the counting of each sample, tables were used to give factors for correcting the counting rate to that which would have been obtained at a standard reference time, usually the time of addition of the tracer.

(d) Self absorption.

In liquid counter tubes a significant amount of radiation is absorbed by the sample itself,

this factor being determined by the density of the liquid. Samples ashed in sulphuric acid had density 1.20 and a self absorption factor of 1.11. All samples were corrected to the counting rate of a solution of density 1.00.

(e) Counter factor.

All counter tubes had been calibrated with ^{32}P and ^{86}Rb standards, and 'counter factors' had been calculated for the conversion of the corrected counts per minute to millimicrocuries of the isotope in question.

9. Statistical Analysis.

The significance of the results was assessed by means of the analysis of variance as outlined by Snedecor and where necessary transformations were made.

10. Calculation of Derived Data.

The quantity of water transpired and the quantities of labelled phosphorus and rubidium in the roots and shoots were measured. From these data it has been found convenient fo calculate the following three quantities.

(a) The transport index is defined as the percentage of the absorbed ions that are translocated to the shoot (Russell and Martin 1953), i.e.

$$\text{Transport Index} = \frac{\text{Shoot content of absorbed ion}}{\text{Total Plant content of absorbed ion}} \times 100$$

(b) The concentration of the nutrient in the transpiration stream, and

(c) The "transpiration stream concentration factor".

Since the upward transport of nutrients from the root occurs largely if not entirely in the transpiration stream in the xylem (Stout and Hoagland 1939; Mason and Phillis 1940), the mean concentration of the nutrient in the transpiration stream can be calculated from the quantity which reaches the shoot and the quantity of water transpired, i.e. Concentration of nutrient in the transpiration stream = $\frac{\text{Shoot content of nutrient}}{\text{Quantity of water transpired}}$

It has been shown by Loughman and Russell (1957), Tolbert and Wiebe (1955), that phosphorus is present in the xylem mainly in the inorganic state, and it may be assumed that rubidium is present in the xylem as free rubidium ions. Thus a comparison of the concentration of the ion in the transpiration stream with the concentration in the

external medium, provides a convenient measure of whether the ion in question moves across the root at the same rate as water. For the purpose of comparison the ratio of the concentration of the ion in the transpiration stream to the concentration of that ion in the external medium has been calculated. This is referred to as the "transpiration stream concentration factor" (T.S.C.F.).

$$\text{i.e. T.S.C.F.} = \frac{\text{Concn. of ion in transpiration stream}}{\text{Concn. of ion in external medium}}$$

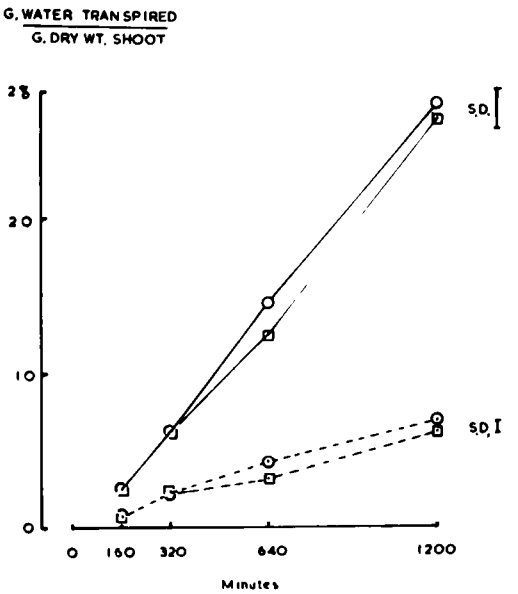
III. EXPERIMENTAL RESULTS

PART I. The Relationship between Transpiration and Salt Uptake.

1. The interpretation of the effects induced by shading.

The primary object of the investigation was to study the effect of transpiration on salt uptake. A simple method of reducing the transpiration rate is by shading, partially or entirely. Fig. 1 shows a typical result of an experiment in which the rate of transpiration of plants placed in the dark was reduced by a factor of four. The use of shading to assess the effects of variation in transpiration on salt uptake is however only valid if it can be assumed that the variations in exposure to light affect salt uptake primarily through transpiration. Two other mechanisms, other than changes in transpiration, whereby shading might affect salt uptake are changes in the carbohydrate level of the plants and changes in the distribution of auxins. The maintenance of plants in the dark for prolonged periods, would be expected to have a considerable effect through both mechanisms. To obviate such effects the use of short experimental periods, normally not longer than 24 hours, was planned.

Figure 1.



The effect of shading on the amount of water transpired (expressed per gram dry weight of shoot) by barley plants. (Expt. 1).

Key to treatments :-

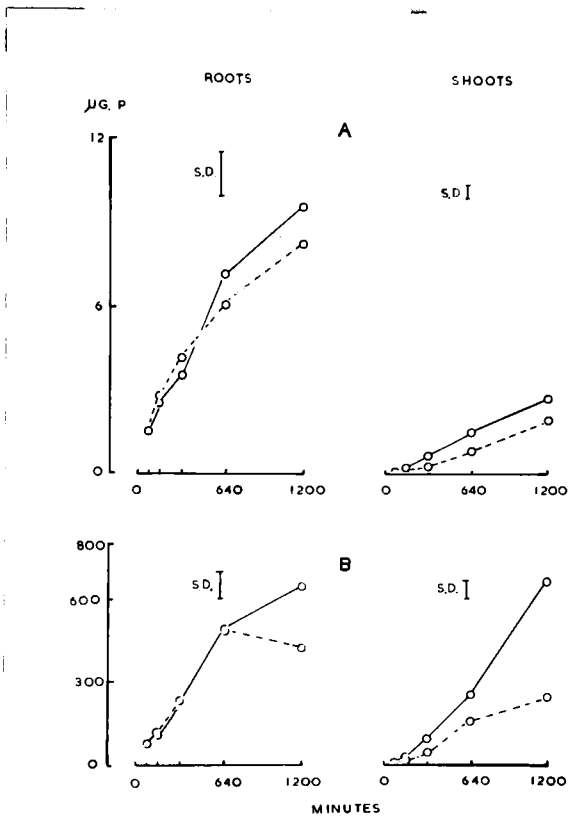
O—O	0.1 p.p.m. P., Light
O···O	0.1 p.p.m. P., Dark
□—□	31 p.p.m. P., Light
□- -□	31 p.p.m. P., Dark

The primary object of preliminary experiments was to determine whether the effects on the uptake of the nutrient, observed in such periods, could be validly attributed to variations in transpiration.

Experiment 1.

Young barley plants of low phosphate status, which had been grown in the greenhouse under natural conditions, were treated with two concentrations of labelled phosphorus, 0.1 p.p.m. and 31 p.p.m. P, for periods of 80, 160, 320, 640, and 1,200 minutes in both the light and dark. Replication was fourfold. The amounts of phosphorus absorbed by the roots and the shoots was determined. The results, which are shown in Fig. 2 indicate that the amount of phosphorus absorbed by plants supplied with 0.1 p.p.m. P was reduced only to a small extent by the dark treatment. Following the initial period the rates of absorption of plants treated in both the light and dark show comparable trends. If the transfer to the dark had induced any metabolic changes its effect would have been expected to have increased progressively, hence a divergence between the rates of absorption would have occurred with time. Since this did not happen, it is reasonable to assume that any effect of dark is primarily related

Figure 2.



The effect of shading on the absorption of phosphorus, expressed as root and shoot contents, by barley plants. (Expt. 1).

Key to treatments :-

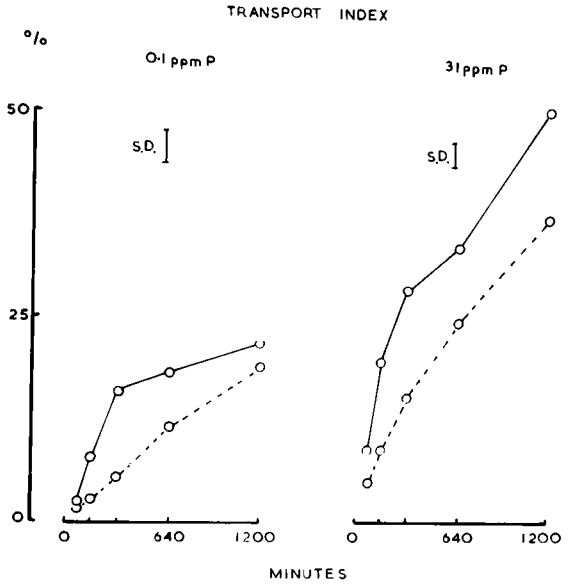
A	0.1 p.p.m. P
B	31 p.p.m. P
O—O	Light
O---O	Dark

to its effects on transpiration, which was constant throughout the whole experiment (Fig. 1).

Plants treated with 31 p.p.m. P (Fig. 2) show a different relationship, in that there was a relatively steady divergence between absorption by plants treated in the light and dark; the divergence in the phosphorus content of the shoots is somewhat comparable to the divergence in transpiration (Fig. 1).

Whilst the evidence so far considered suggests that the dark treatment reduced salt uptake by reducing the rate of transpiration, the evidence is not in itself conclusive, and it could be argued that the reduction in salt uptake was due to other factors induced by the dark treatment. Two facts make this appear unlikely, firstly that the phosphorus contents of the roots were relatively similar (Fig. 2), and secondly that the Transport Index (see p. 27)(Fig. 3) showed parallel but different trends, suggesting that basically similar mechanisms were operating, and that the large difference in the Transport Indices, brought about by the light and dark treatment, are attributable to transpiration. Unfortunately no detailed quantitative comparison of the observations can provide further information on this matter, since the phosphate

Figure 3.



The effect of shading on the transport index. (Expt. 1).

Key to treatments :-

Light

Dark

status of the plants in the two treatments changed to different extents, and this must be expected to have some influence on the absorption rate.

As will be shown later, the effects induced by the light and dark treatments, when a relatively high concentration of phosphorus was supplied, were generally observed, and it was felt that a convenient way of obtaining further information as to whether this effect could be attributed to other factors than transpiration was by the pretreating of plants in the light and dark and then comparing their absorption of phosphorus supplied at the high level in the light. If any alteration in carbohydrate supply or auxin distribution of sufficient magnitude to affect absorption were induced by the dark treatment, any such effects would appear.

Experiment 2.

Barley plants similar to those used in Experiment 1 were pretreated in the light and dark in a phosphate-free solution for 24 hours, before being supplied with a solution containing 31 p.p.m. P and treated in the light for 24 hours. Replication was fourfold.

Inspection of the following figures shows that there was no reduction in the capacity of the plants pretreated in the dark to absorb phosphorus.

		Phosphorus Absorption (μg)		
		Total Plant	Shoot	Root
Pretreatment Light, Treatment Light		583.4	403.2	180.2
" Dark, " Light		646.2	540.7	105.5
S.D. (P = 0.05)		98.1	76.1	44.7

On the basis of the data derived from these two experiments it appears justifiable to assume that variations in transpiration were responsible for the effects induced in plants supplied with 31 p.p.m. P, and other possible effects will be ignored.

Experiment 3.

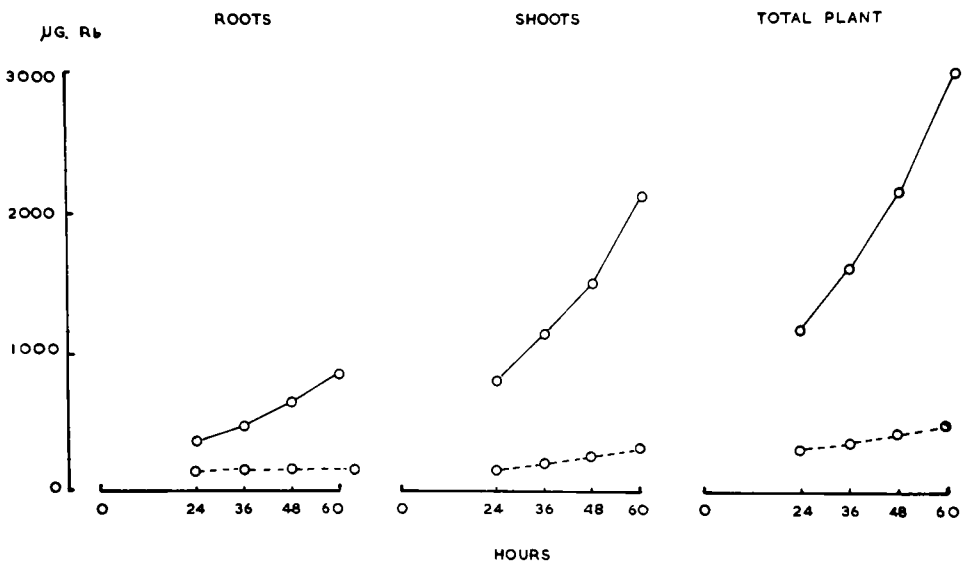
In the experiments with rubidium a different procedure was first adopted in order to ascertain whether the effects of shading on salt uptake could validly be interpreted in terms of the effects of shading on transpiration. Barley plants, supplied with a 'high' level of rubidium (85 p.p.m.) were treated in the light and dark for periods far exceeding the planned experimental times - 24, 36, 48 and 60 hours.

The amount of rubidium absorbed and of water transpired was determined. Fig. 4 shows that dark treatment has markedly reduced the absorption of rubidium, a reduction which shows no progressive divergence. Consideration of Fig. 5 shows that the mean concentration of rubidium in the transpiration stream, calculated from the amounts of rubidium transported to the shoot and the amount of water transpired (see p. 27) was relatively constant over the period investigated.

Experiment 4.

A further experiment similar to Experiment 2, was performed to study the effects of dark treatment on the absorption of rubidium. Barley plants of high potassium status were pretreated in the light and in the dark for 24 hours, before being supplied with a solution containing 85 p.p.m. Rb and treated in the light. Replication was fourfold. Inspection of the following figures shows that there was a slight but not significant, reduction in the capacity of the plants pretreated in the dark to absorb rubidium.

Figure 4.

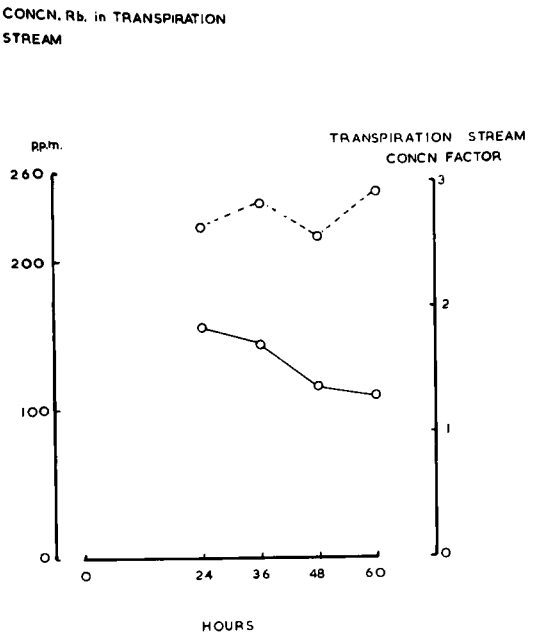


The effect of shading on the absorption of rubidium, supplied at a concentration of 85 p.p.m., by barley plants. (Expt. 3)

Key to treatments :-

Light
Dark

Figure 5.



The effect of shading on the concentration of rubidium in the transpiration stream and on the T.S.C.F. (Expt. 3)

The plants were treated with a solution containing 85 p.p.m. Rb.

Key to treatments :-

O—O Light

O---O Dark

			Rubidium Absorption (μg)		
			Total Plant	Root	Shoot
Pretreatment	Light, Treatment	Light	2061	1076	985
"	Dark, "	Light	2018	1002	956
	S.D. (P = 0.05)		N.S.	N.S.	N.S.

Hence it seems also justifiable with respect to rubidium to interpret the effects of dark treatment on absorption solely in terms of transpiration.

Notwithstanding the apparent justification of this method of varying the transpiration rate of plants, further evidence will be presented later in the results of experiments where, by virtue of the design of the experiment, it is possible to gain more information on this question.

2. The effect of variations in the rate of transpiration on the absorption and distribution of phosphorus by plants supplied with different concentrations of phosphorus.

A. Experiments with Barley plants.

The results of Experiment 1 indicated that the effect of transpiration on the absorption of phosphorus was markedly different at the two levels of phosphorus supplied to the plants. It was thus decided to investigate further into the nature of this differential effect.

Experiment 5.

Young barley plants in the second leaf stage were treated in the light and in the dark for 24 hours, in solutions containing (a) 0.1 p.p.m. P and (b) 31 p.p.m. P. Prior to the experimental treatment the plants had been grown in a phosphate-free solution, thus they were of low phosphate status, but were adequately supplied with other ions. No visible symptoms of nutrient deficiency were observed. All treatments were combined factorily with fourfold replication. The quantity of water transpired and the amount of phosphorus (in μg.) absorbed by the plants in the 24 hours was determined. The

data for phosphorus contents refer to the phosphorus absorbed during the treatment period only.

Results.

The primary data are presented in Table 2, together with the following derived quantities :

(a) the "transpiration stream concentration factor" (T.S.C.F.),

(b) the transport index.

Definitions of the above terms can be found on p. 27.

Since the light treatment caused a significant, but small increase in the dry weight of roots and shoots, the phosphorus contents are expressed both as content of tissue (i.e. in μg. per root and shoot), and per unit dry weight. For the high phosphate treatment both modes of expression show the same result, the phosphorus content of both roots and shoots was significantly increased by a high rate of transpiration. Thus it is concluded that the greater absorption of phosphorus by the plants treated in the light was not in consequence of their growth. At the low level of phosphate, somewhat contrasting effects of transpiration were revealed when the phosphorus content is expressed in μg. and on a dry weight basis. The quantity of phosphorus in the shoot was unaffected by

TABLE 2.

Experiment 5. The effect of shading on transpiration, the plant dry weights, and the absorption and distribution of phosphorus by barley plants, supplied with 0.1 p.p.m. P and 31 p.p.m. P, and treated for 24 hours.

	0.1 p.p.m. P		31 p.p.m. P		S.D. (P = 0.05) 0.1 p.p.m.P	S.D. (P = 0.05) 31 p.p.m.P
	Light	Dark	Light	Dark		
Transpiration-g. water	9.3	0.7	8.4	0.8	1.0	
Dry Weight- mg.						
Root	34.5	27.0	34.2	26.7	3.3	
Shoot	113.7	89.2	117.5	90.7	12.8	
Phosphorus content- μg.						
Root	9.6	7.5	123.0	62.1	0.7	53.9
Shoot	11.5	11.7	290.6	79.8	N.S.	63.3
Total Plant	21.1	19.2	413.6	141.9	1.6	
Phosphorus content- μg. g.						
Root	279.4	273.6	3563.1	2336.6	N.S.	
			3.5518	3.3637		0.167
Shoot	101.2	130.6	2480.1	881.7	11.5	438.8
T.S.C.F.	12.3	155.7	1.1	3.3		
	1.090	2.192	0.045	0.518	0.071	
Transport Index %	54	61	70	56	5.8	

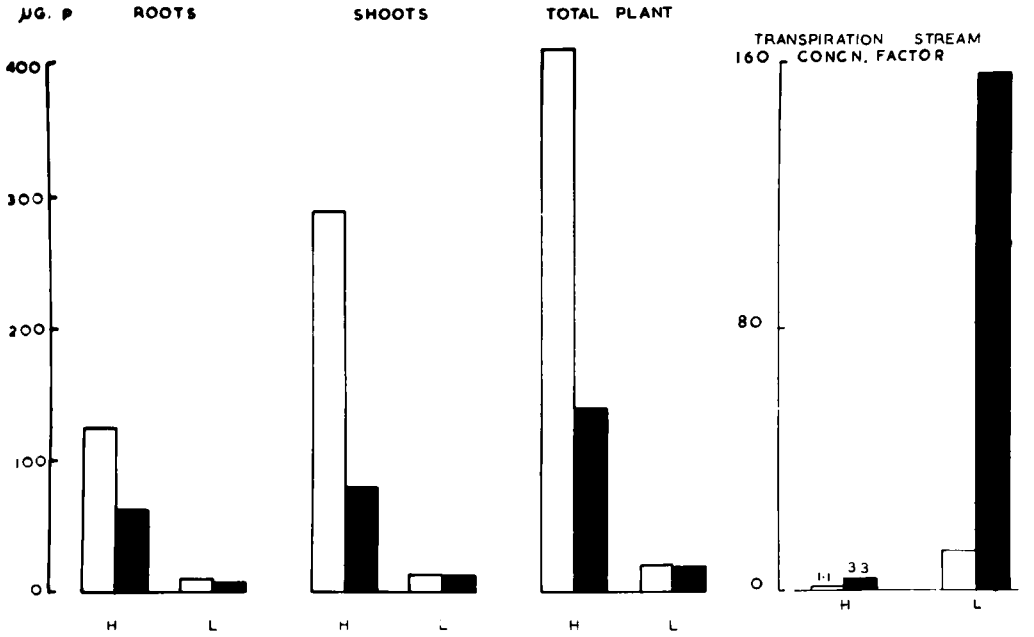
When a logarithmic transformation was necessary for statistical analysis the transformed values are underlined.

the rate of transpiration, the weight change resulting in an increased concentration in the shoots of plants treated in the dark; in the roots the phosphorus content was significantly greater in the plants transpiring at the high rate, whilst the concentrations of phosphorus in the roots did not differ significantly. From this it is concluded that the higher phosphorus contents of the roots of plants treated in the light can be attributed to an increase in dry weight, and that the changes in the phosphorus contents of the shoot cannot be correlated to growth.

Thus the effects of shading on growth which occurred during the course of the treatment should not invalidate the conclusion based on the relationship between the rate of transpiration and uptake.

From Table 2 it is seen that the dark treatment has markedly reduced the rate of transpiration; plants in the light transpired ten times the amount of water transpired by plants in the dark. A large effect of the concentration of phosphorus in the external medium on uptake is observed, Fig. 6. Plants supplied with 31 p.p.m. P absorbed approximately twenty times more phosphorus than plants supplied with 0.1 p.p.m. P when treated in the light; the corresponding figure for the

Figure 6.



The effect of shading on (a) the absorption of phosphorus by barley plants, and (b) the 'transpiration stream concentration factors'. (Expt. 5).

The plants were treated for 24 hours.

Key to treatments :-

H	31 p.p.m. P	
L	0.1 p.p.m. P	
Unshaded column		Light
Shaded column		Dark

(p. 38).

difference in the amount of phosphorus absorbed by plants treated in the dark is seven. The level of phosphorus had only a small effect on the rate of transpiration either in the light or in the dark, Table 2. Thus the relationship between transpiration and uptake is markedly different for the two levels of phosphorus. When the external level of phosphorus was high, a high rate of transpiration resulted in a much greater uptake of phosphorus, whereas at the lower level of phosphorus no such marked effect was observed, and absorption was to a very large extent independent of the transpiration rate.

The T.S.C.F.'s (Table 2 and Fig. 6) show two interesting features. Firstly, that in plants supplied with the high level of phosphorus a reduction in the rate of transpiration resulted in a threefold increase in the concentration of phosphorus in the transpiration stream, whereas the corresponding figure for the plants supplied with the low level of phosphorus was thirteen. This indicates that the translocation of phosphorus to the shoots bore a closer relation to transpiration in the plants supplied with the high level of phosphorus, although at neither level of

phosphorus was the amount of phosphorus translocated to the shoot proportional to the amount of water transpired.

Secondly, that except in the plants supplied with the high level of phosphorus and transpiring at the high rate the T.S.C.F's exceeded unity to a highly significant extent.

Results very similar to those of Experiment 5 are obtained from two further experiments with young barley plants.

Experiment 6. Concentration of phosphorus supplied to the plants was 0.1 p.p.m. Treatment period 20 hours.

Experiment 7. Concentration of phosphorus supplied to the plants was 31 p.p.m. Treatment period 24 hours.

The plants were treated in the light and dark, with fourfold replication in Experiment 5 and eightfold replication in Experiment 6. The absorption of phosphorus during the treatment period was measured and is expressed as μg. Phosphrus.

From Table 3 it is evident that, as in Experiment 4, a high rate of transpiration has only markedly increased the absorption of phosphorus when the concentration of phosphorus supplied was 31 p.p.m. and has had only a small effect when the plants were supplied with 0.1 p.p.m. P.

The contrasting magnitude in the T.S.C.F's induced by shading is also shown by a comparison of Experiments 5 and 6, where in the light and in the dark respectively the T.S.C.F's were 24 and 108 in Experiment 5, and 0.6 and 2.3 in Experiment 6. As in Experiment 4, in the plants supplied with the low level of phosphorus, the T.S.C.F. exceeded unity to a highly significant extent, whereas in the plants supplied with the high level of phosphorus, the amount of phosphorus translocated to the shoot bore a closer relation to transpiration. The T.S.C.F's in the plants supplied with the high level of phosphorus and transpiring at the high rate is significantly less than one. However, it is unwarranted to compare the relative extent to which the T.S.C.F. was effected by the rate of transpiration in Experiments 5 and 6, since there were marked differences in the effect of shading on transpiration in the two separate experiments. In

TABLE 3.

Experiments 6 and 7. The effect of shading on transpiration, the plant dry weights, and the absorption and distribution of phosphorus by barley plants supplied with 0.1 p.p.m. P (Experiment 6) and 31 p.p.m. P (Experiment 7). The plants were treated for 20 hours in Experiment 6 and for 24 hours in Experiment 7.

	Experiment 6 0.1 p.p.m. P		S.D. (P=0.05)	Experiment 7 31 p.p.m. P		S.D. (P=0.05)
	Light	Dark		Light	Dark	
Transpiration-g. water	2.4	0.4		7.3	0.8	
Dry Weight-mg.						
Root	25.7	22.0	2.5	30.2	25.5	3.0
Shoot	86.2	76.7	N.S.	108.5	89.9	9.8
Phosphorus content-μg.						
Root	7.2	5.7	0.8	82.4	47.7	
Shoot	5.7	4.6	N.S.	141.6	59.6	
Total Plant	12.9	10.3	2.0	224.0	107.3	
T.S.C.F.	24	108		0.6	2.3	0.3
Transport Index %	44	44		63	55	3.2

Experiment 5 dark treatment reduced the rate of transpiration by a factor of 6, whereas the corresponding figure in Experiment 6 was 9. These differences are not surprising in view of the different weights of the plants.

Thus it is concluded from Experiment 4, 5 and 6 that the absorption of phosphorus by barley plants is, to a very large extent, independent of the rate of transpiration, and that it is impossible, in the light of (a) the very high T.S.C.F's in the plants supplied with the low level of phosphorus, and (b) the T.S.C.F. less than unity in Experiment 6, to envisage the passive movement of ions across the root in a water stream. However, it appears that the absorption and translocation of phosphorus bears a closer relationship to transpiration when a high level of phosphorus is supplied to the plants.

B. Experiments with Sunflower plants.

Having observed the marked effect of a high rate of transpiration in increasing the absorption of phosphorus supplied at the 'high level' by barley plants, it was decided to study the absorption and distribution of phosphorus by sunflower plants under

similar conditions, in order to determine whether the effects observed were specific to barley or were capable of more general application.

Experiment 8.

Young sunflower plants were treated for 20 hours in the light and in the dark with solutions containing 0.1, 1.0, 2.0, 10.0 and 31 p.p.m. P. Replication was threefold and two plants constituted the sampling unit. The amount of water transpired by all the plants was not measured, but a value was obtained by measuring the amount of water transpired by plants treated with the two extreme levels of phosphorus.

Results.

Inspection of the following figures shows that the rate of transpiration of plants treated in the light was five times greater than the rate of transpiration of plants treated in the dark, and that the concentration of phosphorus in the external medium had a relatively small effect on the amount of water transpired. Hence the mean of these values, corrected for the dry weight of the shoots, can be validly used

in calculating the amount of water transpired. The concentration of phosphorus in the transpiration stream and the T.S.C.F's of the plants treated with labelled phosphorus were then calculated from the extrapolated values for the amounts of water transpired.

Transpiration, g. water transpired/g. dry weight shoot

Treatment Concentration	Light	Dark
0.1 p.p.m. P	64.9	11.6
31 p.p.m. P	67.9	12.9

S.D. (P = 0.05) 6.3

Both the primary and derived data are presented in Table 4. Since the light treatment caused significant, but small, increases in the dry weight of roots and shoots, the phosphorus contents are expressed both as content of tissue (i.e. in μg. per root and shoot) and per unit dry weight. In the plants supplied with 0.1 p.p.m. P the quantity of phosphorus in the shoots of the plants transpiring at the high rate is significantly greater than in the plants transpiring at the low rate, whilst the concentrations of phosphorus in the shoot do not differ significantly. Thus it is concluded that the higher phosphorus contents of the shoot could be attributed

TABLE 4.

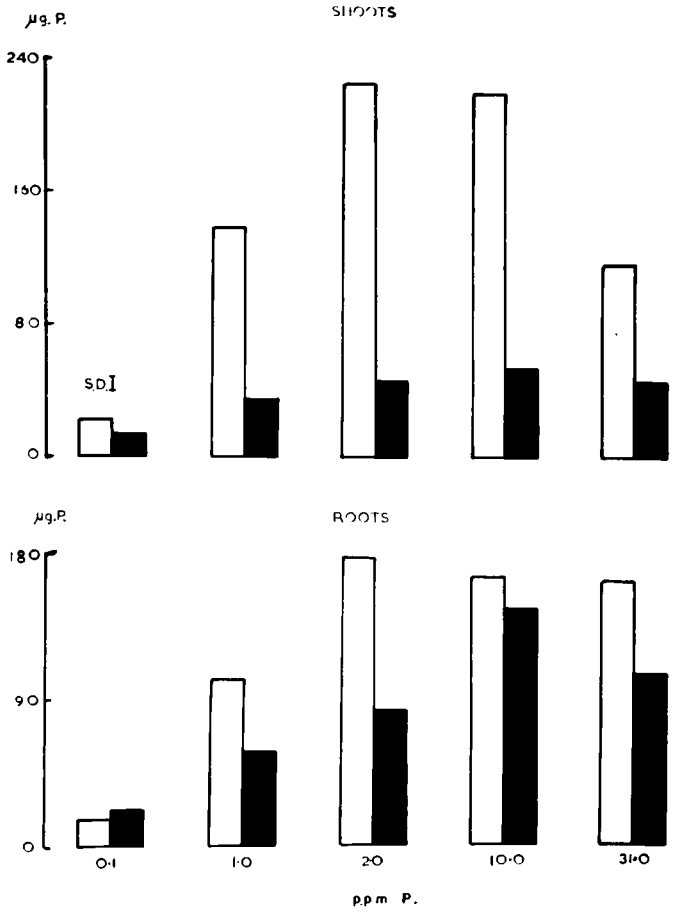
Experiment 8. The effect of shading on the plant dry weight and the absorption and distribution of phosphorus by sunflower plants supplied with 0.1, 1.0, 2.0, 10.0 and 31.0 p.p.m. P and treated for 20 hours.

	0.1 p.p.m.		S.D. P=0.05 (0.1 p.p.m.P)	1.0 p.p.m.		2.0 p.p.m.		10.0 p.p.m.		31.0 p.p.m.		S.D. all concns (P= 0.05)
	Light	Dark		Light	Dark	Light	Dark	Light	Dark	Light	Dark	
Dry Weight- mg.												
Root	45.3	40.0		45.3	40.7	53.0	55.3	42.0	40.7	43.0	39.3	5.8
Shoot	200.3	158.3		192.9	156.3	223.0	212.9	206.0	165.3	180.0	170.6	20.2
Phosphorus content-µg.												
Root	33.4	45.7	N.S.	203.7	115.1	355.9	165.0	332.7	291.4	326.9	212.9	
Shoot	44.4	29.7	8.6	279.7	70.0	458.3	93.3	446.7	109.3	232.7	93.4	
Total Plant	77.8	75.4	N.S.	483.4	185.1	811.2	254.9	779.4	400.7	559.6	306.3	
Phosphorus content-µg/g.												
Root	737	1142	106	4496	2828	6715	2894	7921	7159	7602	5417	
Shoot	222	187	N.S.	1442	447	2005	438	2168	661	1923	547	
T.S.C.F.	34	145		22	35	16	17	3	5	0.6	1.4	
Transport Index %	57.1	39.6		57.7	36.9	56.4	35.2	56.9	27.3	41.6	30.6	12.6

to growth. However, in the plants supplied with the higher levels of phosphorus both modes of expression show the same result; the phosphorus content of the roots and shoots was markedly increased by a high rate of transpiration. Thus the phosphorus contents expressed in μg. will only be considered. The uptake of phosphorus was maximal when the external concentration was 2.0 and 10.0 p.p.m. P; a decrease in uptake was observed in the plants supplied with 31.0 p.p.m. It is evident that the effect of transpiration on absorption was not constant over the range of concentrations employed. At an external concentration of 0.1 p.p.m. P the effect of transpiration on total plant absorption was not significant, but at all other concentrations, particularly at the concentrations where uptake was maximal, a high rate of transpiration has markedly increased the amount of phosphorus absorbed. From Fig. 7 it is seen that the effect of a high rate of transpiration in increasing uptake is more markedly shown by the shoots than by the roots, which suggests that the primary effect of transpiration is one of increasing the rate of translocation.

Table 4 shows that the T.S.C.F's varied in the same manner as was observed in the experiments with barley plants (Fig. 6, Tables 2 and 3), namely that the

Figure 7.



The effect of shading on the absorption of phosphorus (expressed as root and shoot contents) by sunflower plants supplied with 0.1, 1.0, 2.0, 10.0 and 31 p.p.m.P. (Expt. 8).

The plants were treated for 20 hours.

Key to treatments :-

Unshaded column	Light
Shaded column	Dark

T.S.C.F's were high in the plants supplied with 0.1 p.p.m. P (34 in the plants transpiring at the high rate and 145 in plants transpiring at the low rate), and that as the concentration of phosphorus supplied to the plants was increased, the T.S.C.F's decreased. Thus it is concluded that the translocation of phosphorus to the shoot bears a close relation to transpiration only when the level of phosphorus supplied to the plants is high.

Experiment 9.

A further experiment with young sunflower plants was carried out using the two extreme concentrations of phosphorus (0.1 and 31 p.p.m. P) that were employed in Experiment 8. The plants were treated in the light and in the dark for 20 hours. Replication was eightfold.

Results.

The primary and derived data are presented in Table 5, but here it is only necessary to consider the relevant features of the results, in order to compare them with the results of Experiment 8. Large differences in the rate of transpiration occurred, and it is seen that the concentration of phosphorus in the external medium has only had a small effect on the

TABLE 5.

Experiment 9. The effect of shading on transpiration, the plant dry weights, and the absorption and distribution of phosphorus by sunflower plants supplied with 0.1 p.p.m. P and 31 p.p.m. P. The plants were treated for 20 hours.

	0.1 p.p.m.P		31 p.p.m.P		S.D. (P = 0.05)	
	Light	Dark	Light	Dark	0.1 p.p.m.P	31 p.p.m.P
Transpiration-g. water	6.3	1.0	7.3	1.2	1.1	
Dry Weight-mg.						
Root	20.9	16.9	24.1	19.6	N.S.	
Stem	26.2	21.5	29.2	25.4	N.S.	
Leaves	61.5	46.2	70.6	54.6	13.2	
Total Shoot	87.7	67.7	99.8	80.0	19.9	
Phosphorus content-µg.						
Root	6.9	6.6	174.6	61.3	N.S.	
Stem	2.1	1.9	24.1	11.6	N.S.	
Leaves	4.8	2.9	84.5	19.6	0.5	
Total Shoot	6.9	5.0	108.6	31.2	0.8	
Total Plant	13.8	11.6	283.2	92.5	1.1	
Phosphorus content µg/g.						
Leaves	80	68			N.S.	
T.S.C.F.	11	50	0.5	0.9		0.3
Transport Index	50	43	41	32	6.7	

rate of transpiration. Whilst a high rate of transpiration resulted in a large increase in the absorption of phosphorus by the plants supplied with 31 p.p.m. P, only a small but significant increase was observed in plants supplied with 0.1 p.p.m. P. The phosphorus contents of the tissues(in roots, stems and leaves) is expressed in μg. It is only in the case of the leaves of the plants supplied with 0.1 p.p.m. P, where the phosphorus contents when expressed on a dry weight basis show no significant difference between the light and dark treatments, that the difference between the phosphorus contents could be attributed to growth (Table 5).

The calculated values for the T.S.C.F's varied not only with the concentration of phosphorus in the external medium but also with the rate of transpiration. In the plants supplied with the low level of phosphorus the T.S.C.F. in plants transpiring at the low rate was approximately five times greater than in the plants transpiring at the high rate: in the plants supplied with the high level of phosphorus the difference between the T.S.C.F's was just significant. As in Experiment 8, very high T.S.C.F's were observed in the plants supplied with the low level of phosphorus,

whereas in the plants supplied with the high level of phosphorus the T.S.C.F for the plants transpiring at the high rate was significantly less than one.

It is thus concluded from Experiments 8 and 9 that the absorption of phosphorus by sunflower plants is to a very large extent independent of the rate of transpiration, and that some other mechanism than water movement is operative in the transfer of phosphorus from the roots. The very high T.S.C.F's observed in the plants supplied with low levels of phosphorus make it impossible to interpret these results as providing evidence for the passive transport of phosphorus across the root to the vascular tissue.

It is apparent from Experiments 5, 6, 7, 8 and 9 that the effects of varying the rate of transpiration on the absorption and distribution of phosphorus from solutions of different concentrations, are of essentially the same nature in both barley and sunflower plants.

3. The effect of variations in the rate of transpiration on the absorption and distribution of phosphorus by plants of different phosphate status.

The results of Experiments 1, 5, 7, 8 and 9 showed that the rate of transpiration exerted a large effect on the absorption of phosphorus only when the external level of phosphorus was high: when the external level was low, the absorption of phosphorus was either independent of the rate of transpiration or was only slightly affected by it. (Experiments 1, 5, 6, 8 and 9). It was also shown that it was only in the plants supplied with the high level of phosphorus that the T.S.C.F's approached unity. This being so it was decided to study the extent to which the phosphate status of the plants affected the absorption, and particularly the translocation of phosphorus.

A. Experiments with barley plants.

Experiment 10.

Young barley plants of low phosphate status, similar to those used in previous experiments, were pretreated for 48 hours, prior to the period in

which the absorption of labelled phosphorus was measured, in

(a) a phosphate-free solution,

(b) a solution containing 31 p.p.m. P,

(c) a solution containing 93 p.p.m. P.

The plants were then treated in the light and in the dark for 24 hours in solutions containing (a) 31 p.p.m. P and (b) 93 p.p.m. P. All pretreatments and treatments were combined factorily in fourfold replication.

Results.

The amount of phosphorus absorbed during the pretreatment period was measured, and it can be seen from Table 6 that the pretreatment in solutions containing phosphorus increased the phosphorus content of the plants by a factor of two. The phosphorus content of the plants pretreated in phosphate-free solutions is taken as the initial phosphorus content of the seed (560 μg/4 seeds - Proctor). No significant difference occurred between the total phosphorus contents of the plants pretreated with 31 p.p.m. P and 93 p.p.m. P, however, the phosphorus

TABLE 6.

Experiment 10. The absorption and distribution of phosphorus by barley plants supplied with 31 p.p.m. and 93 p.p.m. P during the pretreatment period of 48 hours

	31 p.p.m.P	93 p.p.m.P	S.D. (P = 0.05)
Phosphorus content (μg)			
Root	141.2	228.2	50.7
Shoot	380.0	317.4	N.S.
Total Plant	521.2	545.6	N.S.

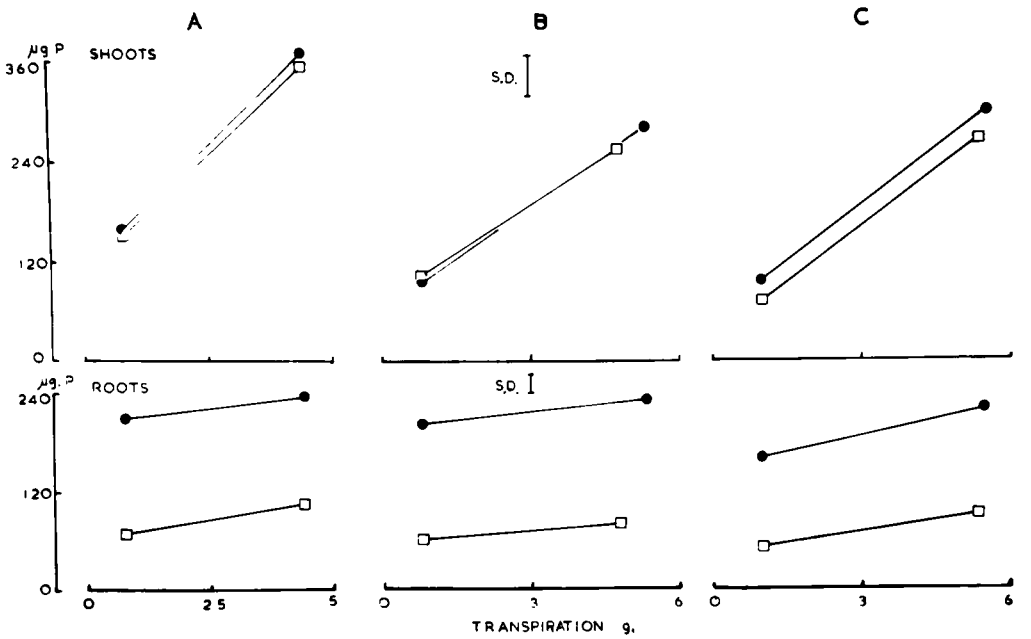
(Initial phosphorus content - 560μg.)

contents of the roots differed significantly; in the plants pretreated with 31 p.p.m. P the phosphorus content of the roots was less than in the plants supplied with 93 p.p.m. P.

The primary and derived data are expressed in Table 7. The plants treated in the light transpired five times the amount of water transpired by the plants treated in the dark. There were small, but significant, differences in plant dry weight. However the phosphorus contents (in μg.) of the tissues varied to a much greater extent than the dry weights. Thus the phosphorus contents expressed both as content of tissue (i.e. in μg. per root and shoot), and per unit dry weight would show the same result. The phosphorus contents expressed in μg. will here be considered. In other experiments where a similar situation occurs, the phosphorus contents will be expressed in μg. and no reference will be made to the phosphorus contents expressed on a dry weight basis.

Table 7, and also Fig. 8, show that the quantity of phosphorus absorbed in the treatment period was inversely related to the concentration of phosphorus supplied in the pretreatment period; the differences

Figure 8.



The effect of shading on the absorption and distribution of phosphorus by barley plants of different phosphate status. (Expt. 10).

Key to treatments :-

A	pretreated in a phosphate-free solution
B	" " " solution containing 31 p.p.m. P
C	" " " " 93 p.p.m. P
□—□	31 p.p.m. P
●—●	93 p.p.m. P

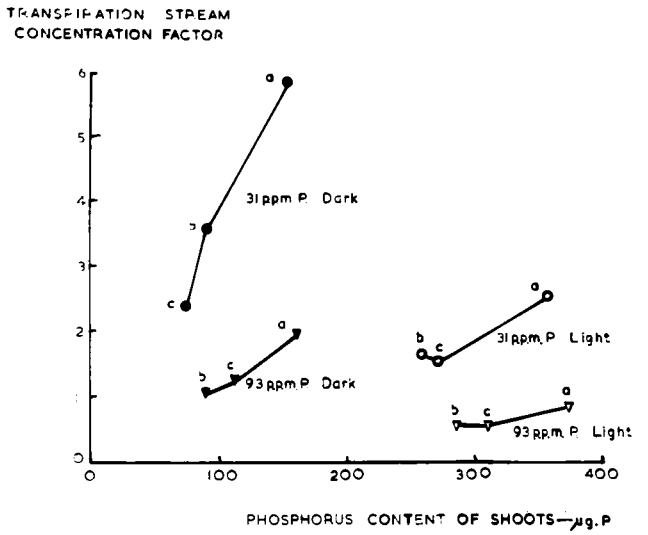
(p. 51)

in the amounts of phosphorus absorbed were, however, small and slightly variable. During the treatment period a high rate of transpiration markedly increased the absorption of phosphorus, an effect which is shown (Fig. 8) strikingly by the phosphorus content of the shoots.

In the plants of high phosphate status that were treated with 31 p.p.m. P, the phosphorus contents of the shoots of the plants transpiring at the high and low rate differed by a factor of 3.6 : the corresponding figure for the plants of low phosphate status is 2.3. Thus the effect of transpiration on the translocation of phosphorus to the shoot was greater in the plants of high phosphate status.

From Table 7 it is seen that the concentration of phosphorus in the transpiration stream varied markedly with the rate of transpiration. It is to be borne in mind that in the calculation of the concentration of phosphorus in the transpiration stream, no account is taken of the phosphorus absorbed during the pretreatment period since it was unlabelled. The effects of pretreatment on the amount of phosphorus translocated and on the T.S.C.F's are compared in Fig. 9 for the four conditions of light and phosphorus concentration employed during the treatment period,

Figure 9.



The relationship between the amount of phosphorus translocated to the shoot and the transpiration stream concentration factor. (Expt. 10).

Key to treatments :-

a. Plants pretreated in a phosphate-free solution.
b. " " in a solution containing 31 p.p.m.P
c. " " " " " " 93 p.p.m.P

by plotting the T.S.C.F's against the amounts of phosphorus translocated to the shoot.

In the plants which showed the greatest translocation during the treatment period (i.e. the plants treated with 93 p.p.m. P in the light), the T.S.C.F. was only slightly affected by the pretreatment. By contrast the plants which translocated the smallest amount of phosphorus in the treatment period (i.e. the plants treated with 31 p.p.m. P in the dark) showed a marked effect of pretreatment on the T.S.C.F. In the plants treated with 93 p.p.m. P in the dark and with 31 p.p.m. P in the light both the amounts of phosphorus translocated and the effects of pretreatment on the T.S.C.F. occupy an intermediate position between the above two extremes.

Thus it appears that it was only in the plants that possessed the largest capacity for translocation that there was a close relationship between transpiration and translocation.

Experiment 11.

In Experiment 10 it was shown that the absorption and translocation of phosphorus supplied at 31 p.p.m. P and 93 p.p.m. P was influenced by the phosphate status of the plants. Thus it was decided

to investigate the effect of phosphate status on the absorption and translocation of phosphorus supplied at the low level of 0.1 p.p.m. P.

Young barley plants in the second leaf stage were pretreated in (a) a phosphate-free solution , and (b) a solution containing 31 p.p.m. P for 18 hours. The amount of labelled phosphorus absorbed was measured after treating the plants in a solution containing 0.1 p.p.m. P for 6 hours in the light and in the dark. All pretreatments and treatments were combined factorily in fourfold replication.

Results.

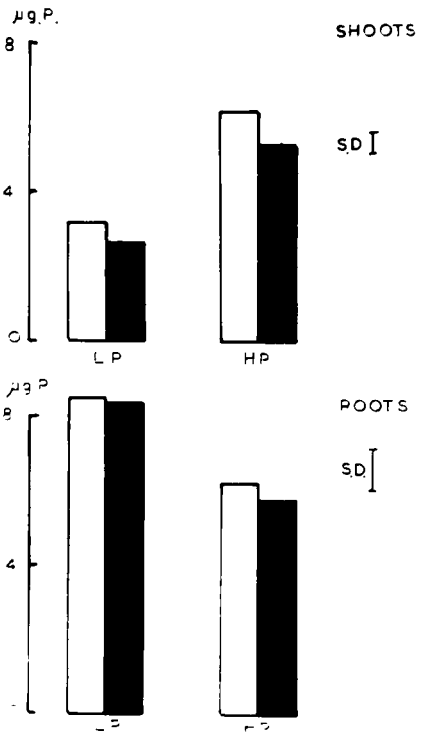
The primary and derived data are given in Table 8. The plants treated in the light transpired four times the amount of water transpired by plants treated in the dark. Absorption of phosphorus by the plants was not significantly effected by the pretreatment or by the rate of transpiration, but the distribution of the absorbed phosphorus was markedly affected by the pretreatment. It is seen from Fig. 10 that the plants pretreated in 31 p.p.m. P translocated a significantly greater quantity of labelled phosphorus to the shoots than the plants pretreated in phosphate-free solutions.

TABLE 8.

Experiment 11. The effect of shading on transpiration, the plant dry weights, and the absorption and distribution of phosphorus by barley plants of different phosphate status. The plants were treated for 6 hours in a solution containing 0.1 p.p.m. P.

Pretreatment Concn. P	0		31 p.p.m.		S.D. (P = 0.05)
	Light	Dark	Light	Dark	
Transpiration-g. water	2.6	0.6	2.6	0.6	
Dry Weight-mg.					
Roots	47	42	42	43	N.S.
Shoot	155	144	157	156	N.S.
Phosphorus content-μg.					
Root	8.5	8.3	6.1	5.7	1.6
Shoot	3.1	2.7	6.1	5.3	0.9
Total Plant	11.6	11.0	12.2	11.0	N.S.
T.S.C.F.	12	40	24	88	
Transport Index %	27.1	24.3	50	48	

Figure 10



The effect of shading on the absorption of phosphorus by barley plants of different phosphate status. (Expt.11).

The concentration of phosphorus supplied to the plants during the 6 hour treatment period was 0.1 p.p.m. P

Key to treatments :-

LP Plants pretreated in phosphate-free solution
HP " " " solution containing 31 p.p.m.P

Shaded column Dark
Unshaded column Light

In both the high and low phosphate status plants the T.S.C.F's (Table 8') in the plants transpiring at the low rate were three to four times greater than in the plants transpiring at the high rate. Thus it appears that transpiration had a similar effect on translocation regardless of the phosphate status of the plants and that the higher transport indices of the high phosphate status plants was due solely to the pretreatment. This latter observation is in agreement with the results of Russell, Martin and Bishop (1953). The T.S.C.F's were in excess of unity, particularly in the plants of high phosphate status where the retention of phosphorus in the roots was reduced.

It is concluded from Experiments 10 and 11 that, in the young barley plants used, it is only when very large amounts of phosphorus are translocated that a close relationship is observed between transpiration and translocation. Although a detailed comparison of Experiments 10 and 11 is not possible owing to the differences in the plant dry weights and the rates of transpiration, it is noted that in Experiment 10 the amounts of phosphorus translocated varied from 74 to 373 μg, whereas in Experiment 11 the amounts of

phosphorus translocated varied from 2.7 - 6.1 μg.

B. Experiments with Sunflower plants.

Experiment 12.

Young sunflower plants were treated for eight days, prior to the period in which the absorption of phosphorus was measured, in (a) phosphate-free solution and (b) in solutions containing 31 p.p.m. P.

The plants were then treated in a solution containing 31 p.p.m. P, in the light and in the dark for 24 hours. Pretreatments and treatments were combined in sixfold replication.

Results.

Inspection of Table 9, in which the primary and derived data are presented, shows that the plants treated in the light transpired approximately five times the amount of water transpired by the plants treated in the dark; the pretreatment of the plants had only a slight effect on transpiration. Small differences in plant dry weight occurred, whilst the differences in the amounts of phosphorus absorbed were very large. The phosphorus contents expressed

TABLE 9.

Experiment 12. The effect of shading on the transpiration, the plant dry weights, and the absorption and distribution of phosphorus by sunflower plants of different phosphate status. The plants were treated for 24 hours in a solution containing 31 p.p.m. P.

Pretreatment Concn. P	0		31 p.p.m.		S.D. (P = 0.05)
	Light	Dark	Light	Dark	
Transpiration-g. water	8.2	1.5	8.7	1.9	
Dry Weight- mg.					
Root	37.5	25.8	33.8	28.8	N.S.
Shoot	107.3	78.2	99.8	85.3	19.4
Phosphorus content- μg.					
Root	502.2	342.1	267.5	95.8	
Shoot	591.4	241.9	196.6	37.3	109.0
Total Plant	1093.6	584.0	464.1	133.1	122.0
T.S.C.F.	2.3	4.9	0.7	0.6	
	1.36	1.67	0.83	0.78	0.18
Transport Index %	54.1	41.4	42.4	28.0	7.4

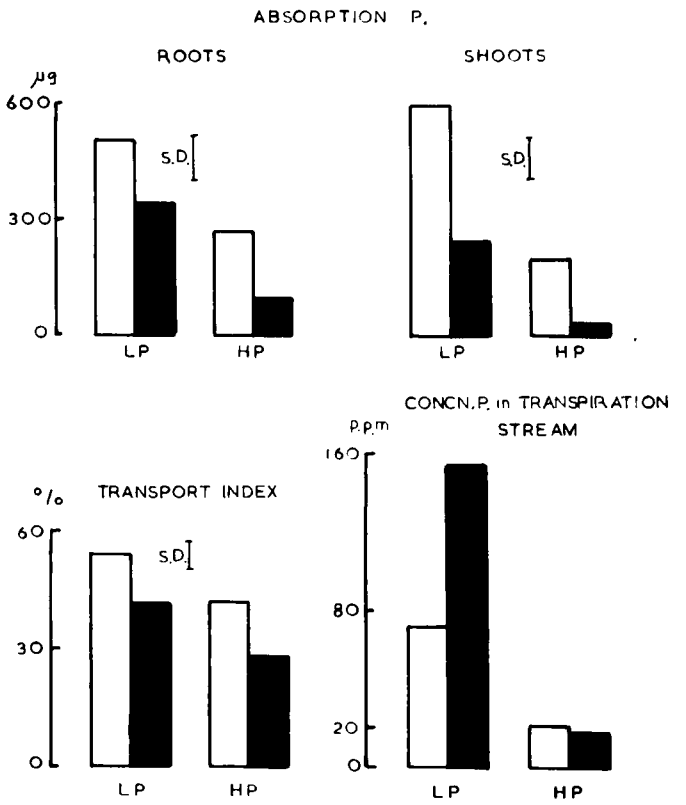
When a logarithmic transformation was necessary for statistical analysis, the transformed values are underlined.

in μg. will here be considered.

From Table 9 it is seen that the plants transpiring at the high rate absorbed a markedly greater quantity of phosphorus than plants transpiring at the low rate; in the plants of high phosphate status, the amounts differed by a factor of 3.5 : the corresponding figure for the plants of low phosphate status is 1.9. Thus the effect of transpiration was greater in plants of high phosphate status.

The greater absorption by the plants transpiring at the high rate was shown to a greater extent by the increase in phosphorus contents of the shoots than of the roots (Fig. 11). The T.S.C.F's (Table 9) shows that in the plants of low phosphate status the concentration of phosphorus in the transpiration stream was greater than in the external medium: in the plants of high phosphate status the concentration of phosphorus in the transpiration stream was less than in the external medium. The rate of transpiration significantly affected the T.S.C.F. only in the low phosphate status plants. Thus it appears that in the plants of high phosphate status there was a close relationship between translocation and transpiration. However the T.S.C.F. significantly less than unity indicates that even in the high

Figure 11.



The effect of shading on the absorption and distribution of phosphorus by sunflower plants of different phosphate status. (Expt. 12).

The plants were supplied with 31 p.p.m. P.

Key to treatments :-

LP plants pretreated in phosphate-free solutions
HP " " " solution containing 31 p.p.m.P
Unshaded column Light
Shaded column Dark

phosphate status plants the rates of water and phosphorus transport through the plant were different. Thus the results cannot be interpreted as providing evidence for the passive transport of phosphorus across the root in a water stream.

4. The effect of variations in the rate of transpiration on the absorption and distribution of rubidium by plants supplied with different concentrations of rubidium.

Having observed the large effect that transpiration exerted on the absorption of phosphorus by both barley plants and sunflower plants when the external concentration of phosphorus was high, it was decided to investigate the absorption of rubidium by the same plant material using equivalent concentrations of rubidium.

A. Experiments with Barley plants.

Experiment 13.

For this experiment barley plants in the second leaf stage that had been grown in full culture solutions and then in a potassium-free solution for 24 hours prior to the treatment with labelled rubidium, were used. The plants were treated for 20 hours in light intensities of 500 f.c. and 250 f.c. and in the dark, and were supplied with two concentrations of labelled rubidium, 85 p.p.m. and 0.31 p.p.m. Replication was fivefold.

The amount of rubidium absorbed during the treatment period and amount of water transpired by the plants was measured. However, since it was necessary to use large culture vessels (1.5 litre) with the 0.31 p.p.m. treatment to avoid depletion of the treatment solution, no measurement of transpiration could be made. Instead the amount of water transpired by a sub-sample of the plants was measured.

Results.

The primary and derived data are presented in Table 10. The amount of water transpired by the plants treated in the light intensity of 250 f.c. was approximately half that transpired by plants treated in the light intensity of 500 f.c. The transpiration rates of the plants treated in the light (500 f.c.) and in the dark differed by a factor of five. Slight differences in plant dry weight occurred, but it is apparent on inspection of Table 10 that it is only in the roots that the slight differences in rubidium content could be attributed to growth, whereas the differences in the rubidium content of the shoots are very large when compared

TABLE 10.

Experiment 13. The effect of shading on transpiration, the plant dry weight and the absorption and distribution of rubidium by barley plants supplied at 0.31 and 85 p.p.m. Rb. The plants were treated for 20 hours.

	0.31 p.p.m. Rb			85 p.p.m.Rb			S.D. (P=0.05)	
	500 f.c.	250 f.c.	Dark	500 f.c.	250 f.c.	Dark	0.31p.p.m.	85p.p
Transpiration-g. water	4.5	2.8	0.8	4.5	2.3	0.7		
Dry weight- mg.								
Root	35.8	34.8	34.6	40.2	34.0	32.0	3.3	
Shoot	86.2	80.2	79.8	111.0	94.4	93.8	9.5	
Rubidium content -µg.								
Root	183.3	155.9	150.1	733.7	633.8	611.1	30.4	N.S.
Shoot	50.6	37.8	18.5	878.0	795.4	496.7	7.9	288.7
Total Plant	233.9	193.7	168.6	1611.7	1419.2	1107.8	35.1	455.4
Concn. Rb. in the transpiration stream p.p.m.	11.3	13.5	22.6	195.2	352.3	715.5		
T.S.C.F.	36.4	43.5	72.8	2.3	4.1	8.4		
	1.560	1.643	1.856	0.363	0.599	0.887	0.149	
Transport Index %	21.6	19.6	11.0	54.5	55.3	44.8	4.4	

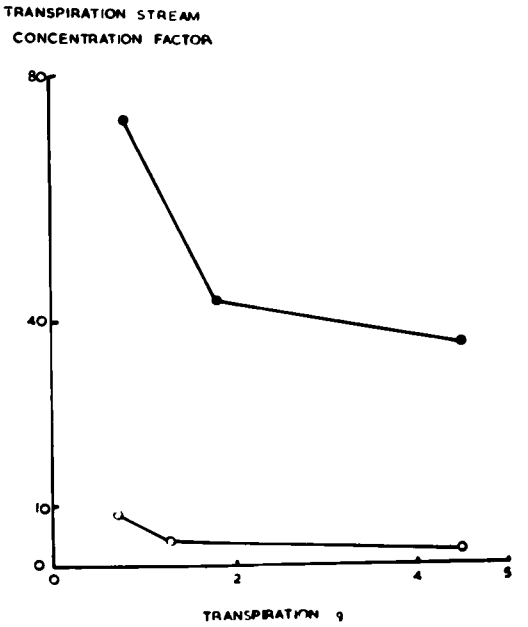
When a logarithmic transformation was necessary for statistical analysis, the transformed values are underlined.

with the differences in dry weight of the shoots. Table 10 shows that, whilst a considerably greater amount of rubidium was absorbed by the plants treated in 85 p.p.m.Rb a high rate of transpiration resulted in a greater uptake at both concentrations of rubidium, than a low rate of transpiration. This effect was shown to a marked extent by the shoot contents whereas the effect of transpiration rate on the root content was very small.

The concentration of rubidium in the transpiration stream (Table 10) was twice as great in plants transpiring at the low rate than at the high rate, in plants supplied with 0.31 p.p.m. Rb; the corresponding ratio for plants supplied with 85 p.p.m. Rb was 3.6. It is to be noted from the T.S.C.F's (Table 10, Fig. 12) that in the plants supplied with the low level of rubidium the concentration of rubidium in the transpiration stream exceeded the concentration in the external medium to a highly significant extent, whereas in the plants supplied with the high level of rubidium the T.S.C.F's more closely approached unity.

Results similar to those of Experiment 13 were obtained from four further experiments using young barley plants, which it is convenient to group together.

Figure 12.



The relationship between transpiration and the transpiration stream concentration factor (T.S.C.F). (Expt. 13)

Key to treatments :-

○—○ 85 p.p.m. Rb

●—● 0.31 p.p.m. Rb

Experiment 14. Concentration of rubidium supplied to the plants was 0.28 p.p.m. Treatment period - 6 hours.

Experiment 15. Concentration of rubidium supplied to the plants was 85 p.p.m. Treatment period - 22 hours.

Experiment 16. Concentration of rubidium supplied to the plants was 85 p.p.m. Treatment period - 24 hours.

Experiment 17. Concentration of rubidium supplied to the plants was 85 p.p.m. Treatment period - 24 hours.

The plants in all experiments were treated both in the light (500 f.c.) and the dark with an additional treatment in the light (250 f.c.) in Experiment 17.

The replication was fourfold in Experiments 14, 16 and 17, and was sixfold in Experiment 15. The primary and derived data are presented in Tables 11 and 12.

In all experiments the plants treated in the light transpired markedly more water than plants

TABLE 11.

Experiments 14 and 15. The effect of shading on transpiration, the plant dry weights, and the absorption and distribution of rubidium by barley plants. In Expt. 14 the plants were treated for 6 hours with a solution containing 0.28 p.p.m.Rb, and in Expt. 15, for 22 hours with a solution containing 85 p.p.m. Rb.

	Experiment 14			Experiment 15		
	0.28 p.p.m. Rb		S.D.	85 p.p.m. Rb		S.D.
	Light	Dark	(P=0.05)	Light	Dark	(P=0.05)
Transpiration-g. water	0.7	0.2		5.7	0.7	
Dry Weight mg.						
Root	21.5	23.5	N.S.	26.8	24.8	N.S.
Shoot	67.2	63.5	N.S.	94.3	77.5	9.5
Rubidium content - μg.						
Root	21.6	20.1	N.S.	655.9	358.5	99.1
Shoot	3.8	1.8	0.5	1746.7	129.7	
Total Plant	25.4	21.9	N.S.	2402.6	488.2	
Concn. Rubidium in transpiration stream p.p.m.	5.5	9.1	2.2	305.1	183.1	52.6
T.S.C.F.	19.6	32.5	7.8	3.6	2.2	0.6
Transport Index %	15.1	8.2		72.5	26.3	

TABLE 12.

Experiments 16 and 17. The effect of shading on transpiration, the plant dry weight and the absorption and distribution of rubidium by barley plants. In both experiments the plants were treated for 24 hours with a solution containing 85 p.p.m. Rb.

	Experiment 16			Experiment 17			
	85 p.p.m. Rb			85 p.p.m. Rb			
	Light	Dark	S.D. (P=0.05)	500 f.c.	250 f.c.	Dark	S.D. (P=0.05)
Transpiration-g.water	5.2	0.7		14.5	10.5	3.1	
Dry weight - mg.							
Root	24.2	23.2	N.S.	57.7	55.5	50.2	N.S.
Shoot	85.7	74.2	8.1	203.2	202.7	186.0	N.S.
Rubidium content- μg.							
Root	359.0	147.2		1076	805	705	30
Shoot	809.2	159.0		985	685	192	
Total plant	1168.2	306.2		2061	1490	897	
Concn. Rubidium in transpiration stream p.p.m.	155.8	223.9	50.3	67.6	65.2	60.4	N.S.
T.S.C.F.	1.8	2.6	0.6	0.8	0.8	0.7	N.S.
Transport Index %	69	52		48	46	21	

treated in the dark, though it is to be noted that the amounts of water transpired varied by different factors in each experiment. Only slight, and in many cases, not significant, differences in plant dry weight occurred. The rubidium contents however differed to a far greater extent than the plant dry weights.

In all experiments, except Experiment 14 where a low level of rubidium was supplied to the plants, a high rate of transpiration caused a marked increase in the absorption of rubidium, and especially on the rubidium contents of the shoots. In Experiment 14 a high rate of transpiration caused a significant increase in the rubidium content of the shoots, although the total quantity of rubidium translocated was very small.

The concentration of rubidium in the transpiration stream of the plants supplied with the low level of rubidium (Experiment 14) was greater in the plants transpiring at the low rate, and markedly greater than the concentration of rubidium in the external medium; the T.S.C.F. for the plants transpiring at the low rate was 32 and for the plants transpiring at the high rate, was 19.

In the other experiments where the level of rubidium supplied to the plants was high, the concentration of rubidium in the transpiration stream was only slightly effected by the rate of transpiration. In Experiment 15 an anomalous result was observed: the T.S.C.F. for the plants transpiring at the high rate was slightly higher than in the plants transpired at the low rate. However, the T.S.C.F's for the plants supplied with the high level of rubidium more closely approached unity than in the plants supplied with the low level of rubidium.

It is concluded from Experiments 13-17 that despite the fact that at the low level of rubidium the rate of transpiration exerted an effect on uptake, the nature of the effect of the rate of transpiration on the uptake of rubidium is essentially the same as the effect of the rate of transpiration on the uptake of phosphorus. For not only was the effect of transpiration on uptake greater when the level of rubidium supplied was high, but also the calculated values for the concentration of rubidium in the transpiration stream varied in a similar manner to that observed in experiments with phosphorus. The T.S.C.F's for plants supplied with the low level of rubidium exceeded unity to a highly significant extent

and were markedly higher in the plants transpiring at the low rate than at the high, whereas in the plants supplied with the high level of rubidium, the T.S.C.F's more closely approached unity and did not differ so markedly with the rate of transpiration.

B. Experiments with Sunflower plants.

Experiment 18.

Young sunflower plants which possessed two pairs of opposite leaves, and had been grown in complete nutrient solutions were treated in the light and in the dark with 85 p.p.m. Rb and 1.34 p.p.m. Rb for 20 hours. The amounts of rubidium absorbed and the amounts of water transpired by the plants was measured. Replication was threefold.

Results.

The primary and derived data are presented in Table 13. The rate of transpiration of the plants treated in the light was six times greater than in the plants treated in the dark. Differences in plant dry weight occurred. However, it is seen that the differences in the rubidium contents of the stems, leaves and roots between the plants treated

TABLE 13.

Experiment 18. The effect of shading on the transpiration, the plant dry weights, and the absorption and distribution of rubidium by sunflower plants treated for 20 hours, and supplied with 1.34 and 85 p.p.m. Rb.

	1.34 p.p.m. Rb		85 p.p.m. Rb		S.D. (P=0.05)
	Light	Dark	Light	Dark	
Transpiration-g. water	33.1	5.0	32.5	5.3	
Dry Weight- mg.					
Root	87.6	71.6	64.0	40.3	23.3
Stem	146.3	101.6	112.3	80.6	38.9
Leaves	274.3	220.9	265.9	149.3	81.5
Rubidium content-μg.					
Root	89.0	39.3	1097	257	
Stem	67.9	11.3	880	145	
Leaves	96.6	8.7	2157	159	
Total Plant	253.5	59.3	4134	561	
Concn. Rb. in Transpiration stream p.p.m.	5.0	3.7	93.7	60.7	
T.S.C.F.	3.7	27	1.10	0.7	
	1.56	1.43	1.03	0.84	0.22
Transport Index %	64.9	33.7	73.5	54.2	

When a logarithmic transformation was necessary for statistical analysis the transformed values are underlined.

in the light and in the dark at each level of rubidium were very large when compared with the differences in plant dry weight.

Considerably greater amounts of rubidium were absorbed by the plants supplied with 85 p.p.m., and it is to be noted that at both levels of rubidium, the plants transpiring at the high rate absorbed more rubidium than the plants transpiring at the low rate. This latter effect is shown to a very marked extent when the rubidium contents of the shoots are compared, indicating that, similar to the effect of transpiration on the absorption of phosphorus, the effect of transpiration is primarily one of affecting the movement of rubidium to the shoot.

The concentration of rubidium in the transpiration stream was only slightly affected by the rate of transpiration at both levels of rubidium. Thus there appears to be a fairly close relationship between transpiration and the translocation of rubidium. However, the T.S.C.F's for the plants supplied with the low level of rubidium were significantly greater than unity, indicating that it is impossible to interpret these results in terms of the passive movement of rubidium across the root in

in a water stream, despite the fact that in the plants supplied with the high level of rubidium the T.S.C.F's did not differ significantly from unity.

5. <u>The effect of variations in the rate of transpiration brought about by changes in the osmotic concentration of the external medium and by shading on the absorption and distribution of phosphorus and rubidium</u>.

A further method of changing the rate of transpiration was employed, namely that of varying the osmotic concentration of the external medium by different concentrations of mannitol. It was thus possible to study the effect of a range of transpiration rates on absorption without introducing any of the possible complicating effects of the dark treatment. Also by treating plants in the dark it was possible to obtain some measure of any effects of the dark treatment other than that of reducing the rate of transpiration. Mannitol was chosen because it is reported to be "an osmotically active but otherwise physiologically inert substance which penetrates into cells with great difficulty" (Collander and Berland 1933).

<u>Experiment 19</u>.

Young barley plants in the second leaf stage and of low phosphate status were treated in the light and in the dark for 24 hours in solutions containing :-

(a) 31 p.p.m. P

(b) 31 p.p.m. P and 0.13 M mannitol

(c) 31 p.p.m. P and 0.26 M mannitol

Replication was fourfold.

Results.

The primary and derived data are presented in Table 14, where it can be seen that the high level of mannitol in the external medium markedly reduced the rate of transpiration both in the plants treated in the light and in the dark. Table 14 shows that the rubidium content of the shoots of plants transpiring at low rates was lower than that of the plants transpiring at high rates. In Fig. 13 the amount of phosphorus translocated to the shoot is plotted against the amount of water transpired. It appears that the translocation of phosphorus bore a linear relationship to transpiration over the whole range of transpiration. Thus it is concluded that the mechanism whereby mannitol and darkness reduced translocation was the same, namely through reducing the rate of transpiration.

Slight differences in plant dry weight occurred. Table 14 shows that mannitol tended to bring about an increase in both the dry weight of the roots and

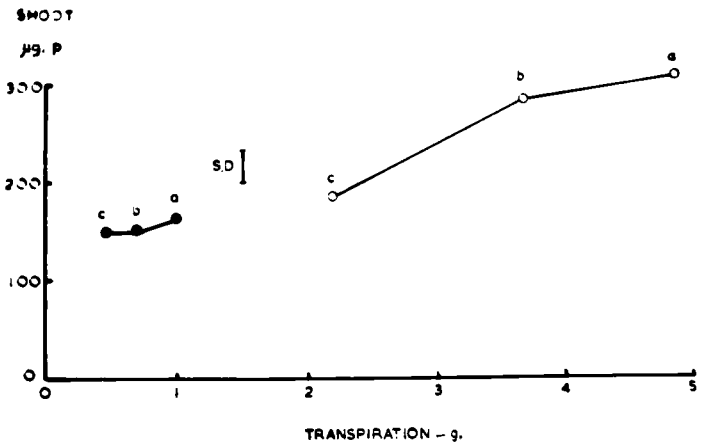
TABLE 14.

Experiment 19. The effect of increasing the osmotic concentration of the external medium by mannitol, and of shading, on transpiration, the plant dry weights and the absorption and distribution of phosphorus by barley plants. The plants were treated for 24 hours in a solution containing 31 p.p.m. P

	Light			Dark			
Concn. Mannitol	Nil	0.13M	0.26M	Nil	0.13M	0.26M	S.D. (P=0.05)
Transpiration-g. water	4.9	3.7	2.2	1.0	0.7	0.6	
Plant Dry weight-mg.							
Root	54	56	61	51	59	63	8.1
Shoot	119	112	108	103	98	103	10.2
Phosphorus content-μg.							
Root	176.5	177.0	188.0	177.0	192.0	234.0	30.5
Shoot	312.0	297.0	183.5	161.0	148.0	146.0	30.5
Total Plant	488.5	474.0	371.5	338.0	340.0	380.0	49.8
T.S.C.F.	2.04	2.59	2.67	5.02	6.69	7.26	
	0.309	0.413	0.426	0.701	0.825	0.861	0.082
Transport Index %	64	63	49	48	44	38	

When a logarithmic transformation was necessary for statistical analysis the transformed values are underlined.

Figure 13.



The relationship between transpiration and the phosphorus content of the shoots of barley plants treated for 24 hours with a solution containing 31 p.p.m. P. (Expt. 19).

Key to treatments :-

●—● Dark
○—○ Light
a. Control
b. 0.13 M mannitol
c. 0.26 M mannitol

the phosphorus contents of the roots. In the plants treated in the dark the increase in root content resulted in the total plant content of the plants transpiring at the lowest rate being greater than in the plants transpiring at the highest rate.

The inverse relationship between transpiration and the T.S.C.F's which was shown by previous experiments was clearly demonstrated. Over the whole range of transpiration rates, an increase in the rate of transpiration resulted in a decrease in the T.S.C.F. The T.S.C.F's of slightly greater than unity were in the same order as those in other experiments where the level of phosphorus supplied to the plant was high.

Experiment 20.

Young sunflower plants that had been grown in complete culture solutions were treated for 24 hours in the light and in the dark in solutions containing :-

(a) 85 p.p.m. Rb

(b) 85 p.p.m. Rb and 0.11 M mannitol

(c) 85 p.p.m. Rb and 0.22 M mannitol

All treatments were combined in fivefold replication.

Results.

The primary and derived data are presented in Table 15. Slight, but not significant, differences in plant dry weight occurred. However, there was a tendency for mannitol to bring about an increase in the dry weight of the roots and also an increase in the rubidium content of the roots (Table 15). This however appears to have had little effect on the relationship between translocation and transpiration. Table 15 shows that the transpiration rates were markedly reduced by treating the plants with mannitol. The high level of mannitol reduced the transpiration rate by factors of 5 and 2.5 for the light and dark treatments respectively.

It can be seen from Table 15 that the rubidium content of the shoots of plants transpiring at low rates was considerably lower than that of the plants transpiring at high rates. When the shoot content is plotted against transpiration (Fig. 14) it is seen that a reduction in transpiration was accompanied by a reduction in translocation to the shoots, regardless of the way in which this effect was induced. The same conclusion is applicable from a consideration of the T.R.C.F. values (Table 15). Thus

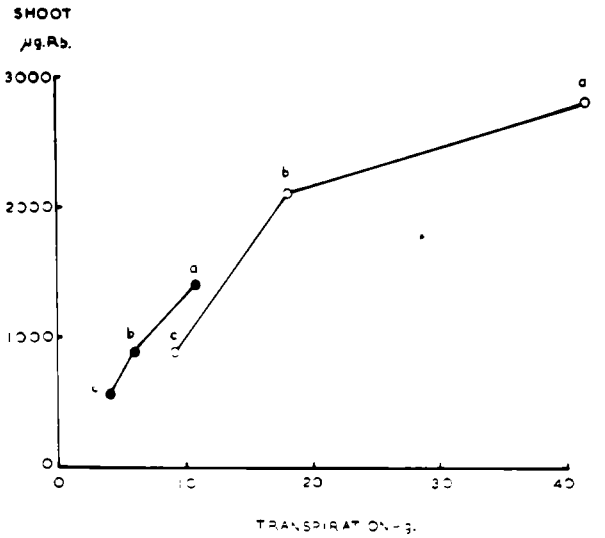
TABLE 15.

Experiment 20. The effect of increasing the osmotic concentration of the external medium by mannitol, and of shading on transpiration, the plant dry weights and the absorption and distribution of rubidium by sunflower plants. The plants were treated for 24 hours in a solution containing 85 p.p.m. Rb.

	Light			Dark			S.D. (P=0.05)
Concn. Mannitol	Nil	0.11M	0.22M	Nil	0.11M	0.22M	
Transpiration-g. water	41.1	17.9	8.7	10.7	6.9	4.3	
Dry Weight-mg.							
Root	204	213	213	169	183	207	N.S.
Shoot	653	586	658	579	540	604	N.S.
Rubidium content-μg.							
Root	2694	3117	2629	2006	2231	2625	
Shoot	2836	2103	828	1398	893	544	
Total Plant	5530	5220	3457	3404	3124	3169	
T.S.C.F.	0.8	1.4	1.1	1.5	1.5	1.7	0.3
Transport Index %	51.3	40.3	24.0	41.1	28.6	17.2	

(p. 71)

Figure 14.



The relationship between transpiration and the rubidium content of the shoots of sunflower plants treated for 24 hours with a solution containing 85 p.p.m. Rb. (Expt. 20).

Key to treatments :-

O—O	Light
●—●	Dark
a	Control
b	0.11 M mannitol
c	0.22 M mannitol

it is concluded that the mechanism whereby mannitol and darkness reduced translocation was the same, namely through reducing the rate of transpiration. Further evidence is thus provided that during the short treatment periods employed, the effect of shading on uptake and translocation can validly be interpreted in terms of a reduction in the rate of transpiration.

6. The effect of shading individual leaves of sunflower plants on the distribution of phosphorus and rubidium in the shoot.

It would be expected from the postulate that the transpiration stream passively transports ions in the vascular tissue that the shading of a single leaf would tend to reduce the quantity of a given ion moving into that leaf solely by reducing the rate of transpiration of the shaded leaf. However, in time it would be expected that the carbohydrate level of the shaded leaf would fall and that this effect per se would reduce the capacity of the leaf to act as a 'sink' for recently absorbed ions. Thus it was decided to study the effect of shading individual leaves on the distribution of phosphorus and rubidium in the shoot for a short period of four hours.

Experiment 21.

Sunflower plants that had been grown in complete culture solutions and which possessed three pairs of leaves were treated for 4 hours in a solution containing 10 p.p.m. labelled phosphorus. The treatment consisted of shading individual leaves on the plants by the muslin shades described on p.20 The four treatments were :-

(1) Control - no leaves shaded,

(2) The two leaves at first node shaded, all other leaves unshaded,

(3) The two leaves at second node shaded, all other leaves unshaded,

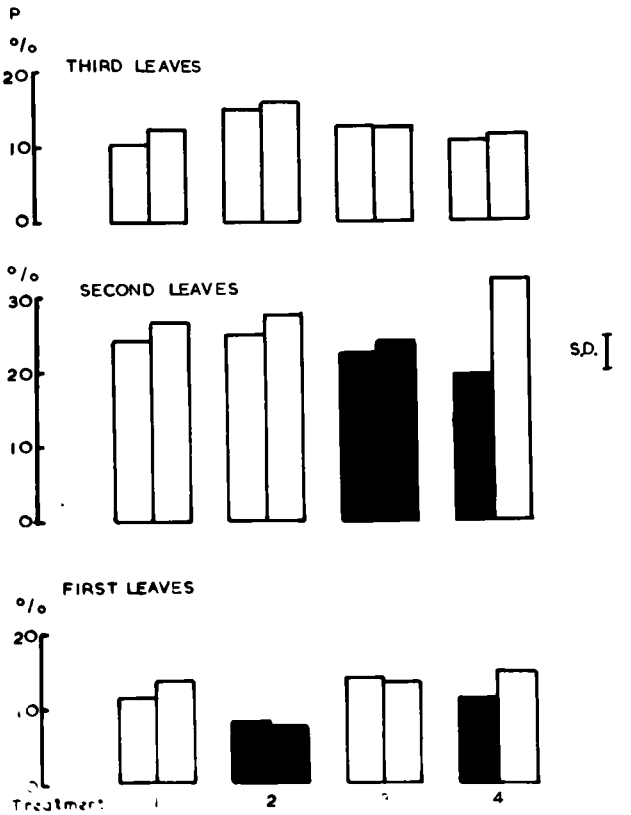
(4) One leaf at first node and one leaf at second node shaded, all other leaves unshaded.

Replication was fourfold.

Only the leaves were harvested and estimation of ^{32}P was carried out by the solid counting of the leaves under an end window tube shielded in a lead castle. The leaves were held between two metal plates, the upper plate having a central circular aperture of diameter 2 cm. The leaves were then placed at a standard distance from the counter tube. The count obtained was an estimate of the amount of absorbed phosphorus per unit area of leaf, and thus by correcting for the area of each leaf, a value was calculated for the total labelled phosphorus content of each leaf. For this preliminary investigation, which was of a qualitative rather than a quantitative nature, it was assumed that the phosphorus in the leaf was uniformly distributed.

The salient data are presented in Fig. 15 where the values for the ^{32}P content of the leaves are

Figure 15



The effect of shading individual leaves on the phosphorus content of the leaves. The phosphorus contents of the leaves are expressed as a percentage of the total leaf content of phosphorus. (Expt. 21).

Key to treatments :-

Shaded column shaded leaves
Unshaded column unshaded leaves

expressed as a percentage of the total ^{32}P content of all leaves on each plant. The shading of only one leaf at a given node reduced the phosphorus content of that leaf when compared with the opposite unshaded leaf. This reduction was more pronounced at the second node than at the first. As stem and roots were not harvested a detailed consideration of the effects of shading a pair of leaves at a node on the overall distribution of the absorbed phosphorus is precluded. However the shading of the two leaves at node, particularly the first node, resulted in a reduction in the phosphorus content of the two shaded leaves.

In conclusion it is to be noted that the effects of the shading of individual leaves on the distribution of phosphorus in the shoot were small compared with the effects of shading the entire shoot on the phosphorus contents of the shoot.

Experiment 22.

Sunflower plants that had been grown in complete culture solutions and which possessed four pairs of leaves were treated for 4 hours in a solution containing 5.8 p.p.m. labelled rubidium. Replication was fourfold - one leaf at the third node and one leaf

at the fourth node were shaded by the muslin shades described on p.20

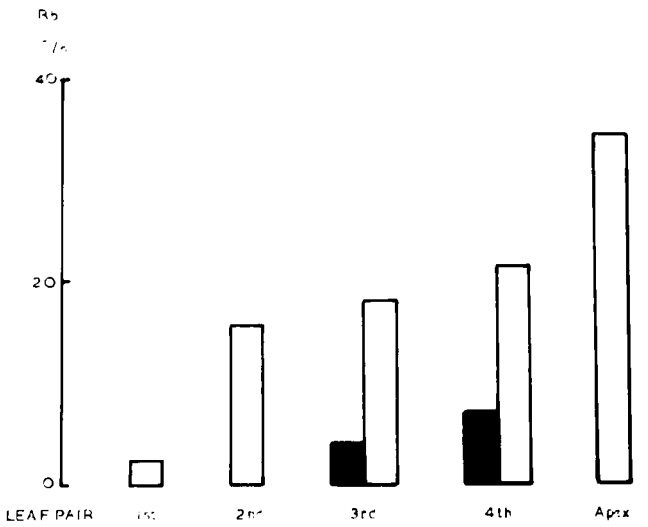
Only the leaves and apices of the plants were harvested. The amounts of rubidium in each leaf, expressed as a percentage of the total rubidium content of all leaves on each plant are shown in Fig. 16

The shading of one leaf at the third and fourth node resulted in a marked reduction in the rubidium content of that leaf when compared with the opposite leaf at the same node. Shading of a leaf at the third node resulted in a reduction in the rubidium content by a factor of five when compared with the rubidium content of the opposite unshaded leaf at the same node; at the fourth node the corresponding reduction in rubidium content by a factor of three occurred.

It is postulated that as a short treatment period was employed, the large effects observed on the movement of rubidium into the leaves are related to the effects of shading on the transpiration of water from the individual leaves.

A detailed comparison of Experiments 21 and 22 is unwarranted owing to the different stages of development of the plants and also because the experiments were carried out at different times in the greenhouse

Figure 16.



The effect of shading individual leaves on the rubidium content of the leaves. The rubidium contents of the leaves are expressed as a percentage of the total leaf content of rubidium. (Expt. 22).

Key to treatments :-

Shaded column	shaded leaves
Unshaded column	unshaded leaves

(The leaves at the first and second nodes, and at the apex were not assayed separately).

where only a limited control of the environment was possible. However it appears that the shading of individual leaves had a greater effect on the distribution of rubidium in the shoot than on the distribution of phosphorus. This effect of shading causing a greater effect on the absorption and the distribution of rubidium was observed in the previous experiments.

PART II. The Effect of Shading Plants for Prolonged Periods on the Relationship between Transpiration and Salt Uptake.

Since the absorption of both phosphorus and rubidium was shown to have been effected in a marked manner by changes in transpiration induced by shading in short experimental periods, consideration was given to the effects of transpiration on uptake which occur when plants are grown continuously under different levels of light intensity. It was, however, only possible to undertake a preliminary examination of this problem.

The experiments were carried out in water culture in the open. Both the plant material used and the method of shading were identical with that of Blackman and Wilson (1951a), (1951b), (1954), and it was thus hoped that it would be possible to link the results of the present investigation with the results of the growth analysis studies of Blackman and Wilson. It was not however possible to determine the growth rates of the plants; this would have required the sampling of plants both before and after the treatment period, and it was not practicable to provide water culture facilities on

this scale. Furthermore in view of the results reported in Part I, it was decided to limit the experimental treatments to 24 hours, which was too short to measure the growth rate.

Experimental Methods.

Plants at the first leaf stage were grown under three light intensities (full daylight (1.00), 0.44 and 0.14 full daylight) for 21 days (the pretreatment period): at the end of this period the plants possessed three pairs of leaves. An experimental treatment period of 24 hours in a labelled phosphate solution under the three levels of light was then imposed. The different light intensities in the pretreatment and the treatment period were combined factorily. The absorption of phosphorus was determined in the 24 hour treatment period only. Details of the experimental procedures, in so far as they differ from those already described, are given below.

(a) Plant Culture

Sunflower plants (variety 'Pole Star') were used in this work. The seedlings were raised in vermiculite, as described on p. 16, until the cotyledons were fully emerged and the first pair of

leaves were visible. The seedlings were then selected for uniformity, the criteria being the size and appearance of the first pair of leaves. They were then transferred to glazed earthenware pots, (Capacity, 13 litres: Internal diameter 10") identical with those used by Blackman and Wilson. The plants were supported on metal covers (Ovenex baking dishes), which were covered with an inert paint, and which were suspended from the lips of the pots (Plate 5). Four plants were supported by each metal cover. This arrangement facilitated the transference of plants from one solution to another. Aeration and circulation of the nutrient solution was effected by means of 'aloxite' blocks connected to an air supply line by glass tubes, which passed through a hole in the center of the metal cover. A complete culture solution (Table 1) was used, and it was not changed during the 21 days before the treatment period. Since iron deficiency in sunflowers, which causes chlorosis, tends to be irreversible despite the rapid accumulation of iron in chlorotic leaves following the application of iron, Jacobson and Oertli (1956), iron was supplied at weekly intervals at the 'quadruple strength' (Table 1).



Culture Vessels used in the experiments described in Part II.

Sunflower plants growing in full daylight.

For explanation see text.

(b) Shading.

As the experiments were carried out in the open, the light intensities referred to are intensities relative to full daylight. The convention adopted is the same as used by Blackman and Rutter (1946) whereby the light intensity of full daylight is always represented by 1.00 even though its absolute value may very considerably between different occasions.

Shading was provided by sheets of perforated zinc supported on light wooden frames (Plate 6). The number and size of the holes varied in the two types of zinc, giving light intensities of 0.44 and 0.14 full daylight. Strips of perforated zinc (width 8") were hung vertically from the edges of the frames to effect lateral shading. The shades were suspended by chains at each corner from iron supports: the height of the shade could thus be readily adjusted as the plants grew. To facilitate handling, the size of each shade was kept small (3' x 4'6"), moreover as was shown by Blackman and Wilson (1951a) a small shade has the advantage that owing to air currents beneath the shade the temperature under the shade and in the open only differ slightly. By placing only five pots under each shade no lateral shading

Plate 6.



Shade of perforated zinc showing the iron supports from which the shade was suspended by chains.

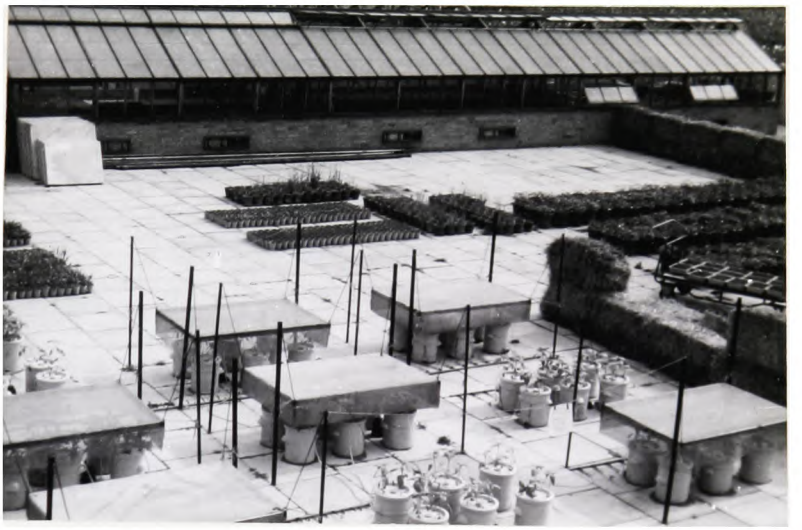
Light intensity beneath shade = 0.44 daylight.

of the plants in one pot by neighbouring plants occurred.

The nine combinations of pretreatment and treatment light intensity with fivefold replication entailed the use of 45 pots, thus in both the pretreatment and the treatment period 15 pots were subjected to each light intensity. These were divided into three groups of 5, and each group was shaded separately. Thus there were nine separate groups of pots. Before transference to the labelled phosphate solutions the 15 pots from each pretreatment light intensity were divided at random into three groups and distributed between the three levels of treatment light intensity.

The site of the experimental area was such that no shading of the pots was caused by neighbouring buildings, the nearest buildings were to the north and east of the area. However, to eliminate any effects due to position, the nine separate groups of pots were arranged in a 3 x 3 Latin Square (Plate 7).

Plate 7.



General view of experimental area showing the layout of the nine groups of pots.

Three groups are subjected to each light intensity.

(c) Harvesting.

At the end of the treatment period the plants from each pot were harvested as a single unit. The procedure for sampling was, firstly, to remove the metal cover and by standing it over an empty pot allow the roots to drain, whilst the leaves (cut at the base of lamina) and the stem (cut just above the highest adventitious root) were collected separately. The roots were washed in demineralised water and dabbed dry between filter paper. Finally all plant samples were placed in oven dishes and dried in an oven for a period of 24 hours at 100°C, and assay of plant samples made in the manner described on p. 22.

(d) Leaf Area Measurement.

On account of the short experimental treatment period it was necessary to harvest the plant samples in a short period of time. Thus for the measurement of leaf areas a quick photographic method was employed. The leaves from each sample as they were harvested were placed on a board fixed at a standard distance from a camera (Shackman Auto Camera) and a photograph taken. A scale was obtained for future reference

by photographing graph paper of known dimensions. The film strip was later rear projected onto thin paper resting on a perspex board and the leaf outline traced. A planimeter set to the appropriate scale was then used to measure the true area of the leaves.

Results.

Experiment 23.

The effect of pretreatment light intensity and treatment light intensity on the absorption and distribution of phosphorus supplied at a concentration of 31 p.p.m. for 24 hours by sunflower plants.

The amount of water transpired by the plants during the treatment period was measured on subsample of the plants by the method described on p. 19. The amounts of water transpired, expressed on a unit leaf area basis were :-

Treatment light intensity (full daylight = 1.00)	1.00	0.44	0.14
Water transpired-g./10 sq.cm/24 hrs.	3.07	2.25	1.90

On the assumption that during the short treatment period the amount of water transpired by the plants in each light intensity was directly proportional to the leaf area, values were calculated for the amounts of water transpired by the plants treated with the labelled phosphate solution (Table 17).

The primary and derived data are presented in Tables 16 and 17. Large reductions in plant

TABLE 16.

Experiment 23. The effect of pretreatment light intensity and treatment light intensity on the plant dry weights, the proportion of root, stem and leaf, the leaf area, the leaf-area ratio, and the leaf area : leaf weight ratio of sunflower plants. Pretreatment period - 21 days, Treatment period - 24 hours.

Pretreatment Light Intensity / Treatment Light Intensity (relative to full daylight (=1.00))	1.00			0.44			0.14			C.D. (P=0.05)
	1.00	0.44	0.14	1.00	0.44	0.14	1.00	0.44	0.14	
Dry weight mg.										
Root	1831	1633	1594	1019	980	766	434	502	407	
	3.254	3.209	3.198	3.006	2.987	2.877	2.636	2.694	2.603	0.028
Stem	1527	1301	1385	1245	1151	935	981	867	751	.247
Leaves	2507	2407	2308	1392	1249	996	740	637	597	
	3.390	3.380	3.361	3.141	3.090	3.000	2.869	2.803	2.771	0.065
Root/Total Plant (x100)	31.2	30.6	30.1	27.9	29.0	28.1	20.1	25.0	23.2	
Stem/Total Plant (x100)	26.0	24.3	26.2	34.0	34.0	34.7	45.5	43.2	42.8	
Leaf/Total Plant (x100)	42.8	45.1	43.7	38.1	37.0	36.2	34.4	31.8	34.0	
Leaf Area (cm^2)	534	560	568	369	349	300	234	219	222	
Leaf Area:Plant Dry Weight ($cm^2/g.$)	91	105	107	101	103	111	109	109	126	11.1
Leaf Area:Leaf Dry Weight ($cm^2/g.$)	213	233	246	265	279	301	317	344	371	

When a logarithmic transformation was necessary for statistical analysis the transformed values are underlined.

(p. 84).

dry weight, expressed as root, stem and leaf dry weights, were caused by shading during the pre-treatment period, whilst shading during the 24 hr. treatment period caused small, and in many cases not significant reductions. The proportion of root, stem and leaf are given in Table 16, and inspection shows that the plants subjected to full daylight in the pretreatment period possessed the largest proportion of roots and the smallest proportion of stem: both observations accord with those of Blackman and Wilson (1954). The plants subjected to full daylight in the pretreatment period also possessed the largest proportion of leaf: this observation does not accord with those of Blackman and Wilson, who observed that shading for 8 days did not significantly affect the proportion of leaf. In the present investigation the period of shading was 21 days, and thus a detailed comparison of the present results with those of Blackman and Wilson is not possible.

Further qualitative comparisons can however be made concerning the effects of shading on the leaf area ratio (Leaf area : Plant weight) and the leaf-area: leaf weight ratio (Table 16). The leaf-area ratio was inversely related to the light

intensity during both the pretreatment and treatment period. These observations are in accord with the many observations made by Blackman and Wilson. The leaf-area ratio was increased by shading during the treatment period to an extent which just attained statistical significance ($P = 0.05$). Thus it appears that certain adaptive changes in the vegetative growth induced by the alterations in light intensity had occurred during the 24 hour treatment period. Blackman and Wilson showed that 8 days after transference from one light intensity to another the leaf-area ratios become adjusted to a new equilibrium irrespective of the large initial differences in the ratio induced by the pretransference intensities.

The effects of shading on the leaf area : leaf weight ratio (Table 16) also agree with the effects observed by Blackman and Wilson: the plants subjected to the lowest light intensity possessed the highest ratios. Shading during the treatment period also tended to increase the ratio.

Table17 shows that shading during the pretreatment period resulted in a large reduction in the amount of phosphorus absorbed during the 24 hour

TABLE 17.

Experiment 23. The effect of pretreatment light intensity and treatment light intensity on transpiration and the absorption and distribution of phosphorus supplied at a concentration of 31 p.p.m., by sunflower plants.
Pretreatment period 21 days. Treatment period 24 hours.

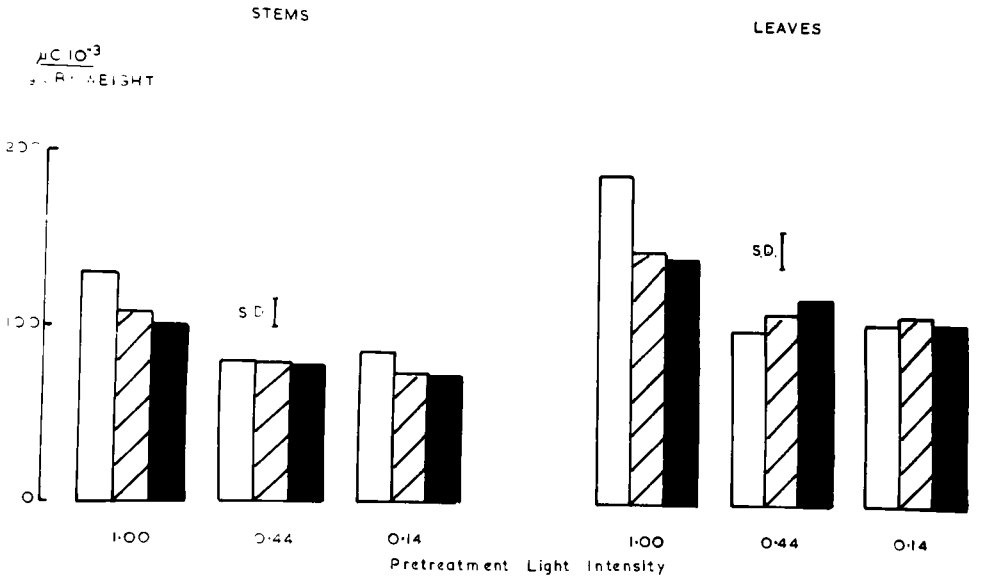
Pretreatment Light Intensity / Treatment Light Intensity (relative to full daylight (=1.00))	1.00			0.44			0.14			S.D. (P=0.05)
	1.00	0.44	0.14	1.00	0.44	0.14	1.00	0.44	0.14	
Transpiration-g. water	163.8	126.1	107.8	113.4	78.5	57.0	71.9	49.3	42.1	
Phosphorus contents (μC10^{-3})										
Root	1849.3	1530.6	1346.7	867.6	865.5	852.7	659.0	619.2	477.5	
Stem	201.5	140.4	142.1	99.9	93.2	73.8	85.3	64.3	55.3	
Leaf	435.0	357.4	335.8	142.2	141.8	120.8	78.8	70.5	63.6	
Total Plant	2485.8	2028.4	1824.6	1109.7	1106.6	1047.3	823.1	754.0	596.4	
Phosphorus contents (μC10^{-3}/g.Dry Wt.)										
Root	998.3	937.3	845.9	865.7	883.2	1152.7	1518.4	1249.4	1153.4	343.7
Stem	131.6	108.7	102.6	80.8	80.7	78.9	87.2	74.2	73.6	18.7
Leaf	173.6	148.2	145.4	102.4	114.9	121.3	106.3	111.0	107.3	22.3
Phosphorus content Leaf (μC10^{-3}/100 cm^2)	81.4	63.8	59.1	38.5	40.6	40.3	33.7	32.2	28.6	
T.S.C.F.	0.77	0.79	0.89	0.43	0.61	0.68	0.45	0.55	0.56	

treatment period. However, owing to the large differences in plant dry weight, the phosphorus content of the tissues, expressed on a dry weight basis, will alone be considered.

From Table 17 it is seen that whilst the concentration of phosphorus in the roots was not reduced by shading the plants during the pretreatment period, the concentration of phosphorus in the stems and leaves was significantly reduced by shading. Thus it appears that whilst the capacity of the roots to accumulate phosphorus was not reduced by shading, the translocation of phosphorus was reduced by shading.

As already discussed, shading during the treatment period reduced the rate of transpiration. The salient results, which are shown in Fig. 17 indicate that it was only in the plants pretreated in full daylight that a reduction in the rate of transpiration caused a significant reduction in the concentration of phosphorus in the stems and leaves. The reduction in translocation was, however, much smaller than the reduction observed in the experiments carried out in the constant environment chambers. In the plants pretreated in 0.44 and 0.14 daylight

Figure 17.



The effect of prolonged shading on the relationship between transpiration and the concentration of phosphorus in the stems and leaves of sunflower plants. (Expt. 23).

Key to treatments :-

☐ High transpiration (Plants treated for 24 hours in full daylight).

☑ Medium transpiration (Plants treated for 24 hours in 0.44 daylight).

■ Low transpiration (Plants treated for 24 hours in 0.14 daylight).

no significant effect of the rate of transpiration on the concentration of phosphorus in the stem and leaves occurred. When the phosphorus content of the leaves is expressed on a unit area basis (Table 17) the same result is shown.

The values for the T.S.C.F.'s were calculated from the labelled phosphorus content of the shoots and the interpolated values for the amounts of water transpired. The concentration of phosphorus in the transpiration stream was maximal in the plants transpiring at the lowest rate. This observation is in accordance with the observation obtained in the experiments carried out in the controlled environment chambers. The concentrations of phosphorus in the transpiration stream was less than the concentration of phosphorus in the external medium. However, a detailed quantitative comparison of these observations is not warranted, as the transpiration values were obtained from a subsample of the plants.

Experiment 24.

The effect of pretreatment light intensity and treatment light intensity on the absorption and distribution of rubidium by sunflower plants.

This experiment was identical with Experiment 23 except that the tracer employed in

the 24 hour treatment period was ^{86}Rb at an activity of 6 μC/litre, with potassium (1 m.eq./litre) as the 'carrier'. The amount of ^{86}Rb absorbed (expressed in millimicrocuries) by the plants was determined.

The amount of water transpired during the treatment period as measured on a subsample of the plants was :-

Treatment light intensity (full daylight = 1.00)	1.00	0.44	0.14
Water Transpired-g./10 sq.cm/ 24 hrs.	1.48	0.85	0.73

The primary and derived data are presented in Table 18. The effects of shading on plant dry weights and on the proportion of root, stem and leaf were very similar to those observed in Experiment 23. Shading during the pretreatment period reduced the proportion of root and leaf and increased the proportion stem.

The leaf ratio and the leaf area : leaf weight ratio were inversely related to the light intensity during both the pretreatment and the treatment period, though in some instances the effect of the light intensity during the treatment period was not statistically significant.

Table 18 shows that shading during the pretreatment period significantly reduced both the dry

TABLE 18.

Experiment 24. The effect of pretreatment light intensity and treatment light intensity on transpiration, the plant dry weights, the proportion root, stem and leaf, the leaf-area ratio, the leaf area:leaf weight ratio, and the absorption and distribution of rubidium by barley plants. Pretreatment period - 21 days. Treatment period - 24 hours.

Pretreatment Light Intensity / Treatment Light Intensity (relative to full daylight (=1.00))	1.00			0.44			0.14			S.D. (P= 0.05)
	1.00	0.44	0.14	1.00	0.44	0.14	1.00	0.44	0.14	
Transpiration- g. water	119.6	67.0	65.4	81.2	42.4	36.2	39.9	22.4	21.7	
Dry Weight mg. Root	1749	1588	1856	811	736	719	270	282	282	
Stem	1613	1393	1715	1192	1035	1180	704	669	734	
Leaves	3786	3315	3847	1858	1689	1604	738	730	759	
Root/Total Plant (x100)	24.5	25.2	25.0	21.0	21.3	20.5	15.8	16.8	15.9	
Stem/Total Plant (x100)	22.6	22.1	23.1	30.9	29.9	33.7	41.1	39.8	41.3	
Leaf/Total Plant (x100)	52.9	52.7	51.9	48.1	48.8	45.8	43.1	43.4	42.8	
Leaf Area:Plant Dry Weight ($cm^2/g.$)	116.8	124.8	126.8	130.5	146.4	141.7	150.8	156.3	179.4	12.2
Leaf Area:Leaf Dry Weight ($cm^2/g.$)	214.7	238.5	244.4	273.1	301.0	310.5	349.6	360.2	409.0	23.9
Rubidium contents ($\mu C10^{-3}$) Root	2517.6	2417.0	2494.5	1277.7	1216.5	1211.5	432.2	446.6	374.8	
Stem	926.5	1315.9	1322.5	578.9	636.2	517.2	325.3	314.2	334.7	
Leaf	1237.2	1298.8	1323.4	470.6	373.1	327.2	119.5	123.5	131.0	
Rubidium contents ($\mu C10^{-3}$/g. Dry Wt.) Root	1454	1545	1361	1565	1659	1684	1603	1580	1341	70
Stem	561	938	785	484	617	440	460	464	457	
Leaf	325	401	351	252	225	203	161	166	174	72
T.[illegible]	3.0	6.5	6.7	2.1	4.0	3.9	1.4	3.2	3.6	

(p. 89).

weight of the plants and the quantity of rubidium absorbed. For this reason the effects are most satisfactorily compared on the plant content per unit dry weight.

From Table 18it is seen that the concentration of rubidium in the stems and leaves but not in the roots, was reduced by shading during the pretreatment period. Thus it appears that whilst the capacity of the roots to accumulate rubidium was not reduced by shading, the translocation of rubidium to the shoots was reduced. Similar effects of shading on the absorption and distribution of phosphorus occurred in Experiment 23. As already shown shading during the treatment period reduced transpiration.

Table 18 shows that the effects of shading during the treatment period on the concentration of rubidium in the stems and leaves were very variable. It is however apparent on comparing the response to treatment light intensity in each group of plants subjected to the same light intensity during the pretreatment period, that a high rate of transpiration was not accompanied by any general increase in the translocation of rubidium to the shoot; some indication of an inverse relationship was apparent.

This is in contrast to Experiment 23 where a high rate of transpiration increased the translocation of phosphorus to the shoot only in the plants pretreated in full daylight.

The values for the T.S.C.F's (Table 18) were calculated from the rubidium content of the shoot and the interpolated values for the amounts of water transpired. The concentration of rubidium on the transpiration stream was inversely related to transpiration in each group of plants subjected to the same pretreatment light intensity. The concentration of rubidium in the transpiration stream was greater, by factors varying from 2 to 7, than the concentration in the external medium. Both the above observations accord with the observations obtained in the experiments carried out in the controlled environment chambers. However, a detailed quantitative comparison of values for the concentrations of rubidium in the transpiration stream is not warranted, as the transpiration values were only obtained from a subsample of the plants. Despite this fact the T.S.C.F's which exceed unity indicate that there was a definite accumulation of rubidium in the transpiration stream.

IV. DISCUSSION

The primary object of this study was to investigate the effect of variations in the rate of transpiration induced by changes in the light intensity on the absorption and transfer of salts to the shoots of intact plants. The absorption of the phosphate and rubidium ions was studied, the use of tracer methods enabled relatively short treatment periods (up to 24 hours) to be employed; thus the effects of growth during treatment were negligible. It was apparent that the effects of transpiration on absorption vary according to the concentration of the ion supplied to the plant, and it was therefore necessary to consider first the effect of transpiration on the absorption of phosphorus and rubidium supplied at different concentrations.

The results which have been presented show that when dilute external concentrations of phosphate were employed, absorption and the transfer of phosphorus from the root to the shoot was either independent of transpiration or only slightly affected

by it. When however the concentration of phosphorus in the external medium was increased a high rate of transpiration resulted in a marked increase in absorption and particularly translocation. (Tables 2, 3, 4, 5; Figs. 2,6&7). With rubidium smaller variations in the relationship between transpiration and absorption were induced as the concentration of rubidium supplied to the plants was varied. The transport of rubidium to the shoots was never independent of the rate of transpiration even at the low concentrations of rubidium employed, but the effect of an increased rate of transpiration was greater when the concentration of rubidium supplied to the plants was high. Qualitatively therefore there is a similarity between the effects of transpiration on the absorption and transport of the two ions: reasons for the quantitative difference will be considered later. The transpiration stream concentration factor (T.S.C.F.)(see p. 27) for all experiments in which there was no pretreatment are summarised in Table 19 . It is apparent that the T.S.C.F's for the plants supplied with the low level of phosphorus or rubidium were greatly in excess of unity, whereas for the plants supplied with the high level of phosphorus and rubidium, the T.S.C.F.

TABLE 19.

Transpiration Stream Concentration Factor

Experiment Number	Treatment concn.	Plant material	Transpiration Stream Concn.Factor		S.D. (P=0.05)
			High rate transpiration	Low rate transpiration	
5	0.1 p.p.m.P	Barley	12	156	
6	0.1 p.p.m.P	"	24	108	
8	0.1 p.p.m.P	Sunflower	34	145	
9	0.1 p.p.m.P	"	11	50	
5	31 p.p.m.P	Barley	1.1	3.3	
7	31 p.p.m.P	"	0.6	2.3	0.3
8	31 p.p.m.P	Sunflower	0.6	1.4	
9	31 p.p.m.P	"	0.5	0.9	0.3
13	0.31 p.p.m.Rb	Barley	36	73	
14	0.28 p.p.m.Rb	"	19	33	
18	1.34 p.p.m.Rb	Sunflower	3.7	2.7	
13	85 p.p.m.Rb	Barley	2.3	8.4	
14	85 p.p.m.Rb	"	3.6	2.2	0.6
16	85 p.p.m.Rb	"	1.8	2.6	0.6
17	85 p.p.m.Rb	"	0.8	0.7	N.S.
18	85 p.p.m.Rb	Sunflower	1.1	0.7	N.S.

more closely approached unity. Moreover, the T.S.C.F's were higher when the net influx of water was small, at both high and low external levels of phosphorus and rubidium. Further evidence of the inverse relationship between the T.S.C.F. and transpiration was provided by the results of Experiments 19 and 20. Plants transpiring at six different rates were obtained by the combination of shading and of changing the osmotic concentration of the external medium. Over the whole range of transpiration rates, the T.S.C.F. was reduced as the transpiration rate was increased (Table 14 and 15) The experiments in which pretreatment with high levels of phosphate was employed (Table 7 , Fig. 9) show that the higher the initial phosphate status of the plants the closer the values of the T.S.C.F's approached unity and the closer was the relationship between transpiration and translocation.

Thus it is apparent that whilst the rate of transpiration can affect the absorption and the pattern of distribution of phosphorus and rubidium, there is no simple relationship between the amounts of water transpired and the amounts of phosphorus and rubidium absorbed or translocated. The high T.S.C.F's observed at low rates of transpiration

are regarded as conclusive evidence that the transfer of salt from the external medium to the vascular sap is dependent on the expenditure of metabolic energy and not on passive transference. Further evidence of the movement of ions by a mechanism independent of the rate of transpiration was provided by the fact that some plants supplied with a high external concentration, and submitted to conditions of rapid transpiration showed T.S.C.F's significantly less than unity.

The existence of metabolic pathways for accumulation does not necessarily exclude the possibility that passive transference can take place, and Hylmo (1953) has sought to explain slightly higher concentrations in the transpiration stream than in the outer medium on the basis that some measure of metabolic uptake may be superimposed upon the passive flow mechanism. It is considered that the present data indicate that the pathways for the passive movement of ions, if they do indeed exist, are of negligible magnitude. The movement of ions along pathways for free diffusion would be dependent on the rate of movement of water and on the difference in concentration between the ends of the diffusion path. Thus if significant pathways for the

diffusion of ions existed, large concentration differences between the internal and external concentration could not be maintained when the rate of water movement across the symplasm is very slow. The high T.S.C.F's observed under these conditions (Table 19) therefore suggests the existence of a barrier against the free diffusion of ions into the xylem. Its effect may be compared to that of the tonoplast of a single cell. Anatomical considerations suggest that the endodermis may be the site of this barrier.

The mechanism whereby ions are transferred across the barrier into the xylem is thus regarded as independent of the rate of transpiration. The alternative interpretation must therefore be considered: that the effect of transpiration on the nutrient content of the shoots is due to the acceleration of the metabolic transport of ions across the root in consequence of the removal, by the transpiration stream, of ions which have been released into the xylem. This interpretation was favoured by Broyer and Hoagland (1943), whose results are in many respects similar to those presented here.

On this basis it would be expected that an

alteration in the rate of transpiration would have a large effect on the transport of ions to the shoot when the removal of ions from the xylem of the root is the rate limiting step. The results of many investigations indicate that this situation is likely to arise when large quantities of ions are entering tissues which are already of a high salt status: Sutcliffe (1952), Humphries (1952), Robertson (1940), Hoagland and Broyer (1942), and Alberda (1948) have shown that plant tissues in general and roots in particular possess a certain capacity for the accumulation of ions, and that the rate of absorption of a given ion is closely dependent upon the concentration of that ion in the tissue. Thus a high concentration as a result of previous absorption will hamper further absorption. Whereas in tissue slices an equilibrium between the nutrient content of the tissue and the external medium is eventually reached and no further absorption occurs, in higher plants the removal of ions, by the transpiration stream from the interior of the root, (i.e. the xylem) can lower the internal concentration so that absorption can continue at a rate dependent upon the rate of removal. The experiments with phosphate here described show that when the

concentration of this ion is sufficiently low the internal concentration becomes a negligible factor regulating the rate of entry, and thus uptake is independent of transpiration. This situation did not occur with rubidium, however with this ion the same trend of decreasing dependence of the transport to the shoot on transpiration became apparent as the concentration was lowered. Since rubidium is unlike phosphorus, in that it is not metabolised into non-ionic forms, and further since a close similarity between the movement of potassium and rubidium occurs, it is not surprising that conditions could not be attained in which the concentration of rubidium within the root ceased to exert some influence on the rate of upward movement. It is considered, therefore, that the quantitative differences in the relationship between transpiration and transport of phosphorus and rubidium to the shoot observed in the experiments accord with the postulate that transpiration affects salt movement only in so far as it lowers the concentration of ions in the xylem and thus accelerates the active transport of ions across the root.

The interpretation here advanced is considered

to be compatible with all the available evidence on the relationship between metabolism and nutrient absorption in intact plants.

The transport of nutrients to the shoots has been observed to be markedly reduced by respiratory inhibitors: Butler (1953b) showed that the transport of chloride to the shoots of young wheat plants was inhibited by dinitrophenol: Brouwer (1954) also observed that both the uptake and translocation of chloride by beans could be reduced by dinitrophenol without a concomitant reduction in the rate of transpiration: Russell, Martin and Bishop (1953) showed that except under exceptional circumstances, respiratory inhibitors markedly reduce the absorption and the transference of ions to the shoots of barley plants. With similar plant material and identical concentrations of inhibitors (Sodiumazide, 2-4 Dinitrophenol and sodium diethyl dutguicarbamate) the author has shown that the rate of transpiration is only slightly reduced by these inhibitors (Table 20). Russell and Martin (Table 21) have also shown that with young barley plants supplied with 10 p.p.m. P sodium azide reduces the transpiration rate by a factor of two, whilst the corresponding figure for the reduction in the amount of phosphorus

TABLE 20.

The effect of inhibitors on transpiration

The concentrations of the inhibitors employed are the same as those employed by Russell, Martin and Bishop (1953).

	Transpiration g. water/18 hours
Control	6.9
Sodium azide (10^{-3}M)	2.7
2:4-dinitro phenol (10^{-5}M)	4.7
Sodium diethyldithiocarbamate (10^{-4}M)	5.9

TABLE 21

The effect of Sodium Azide on transpiration, the plant dry weights and the absorption and distribution of phosphorus by young barley plants supplied with 10 p.p.m. P. (Russell and Martin).

	10^{-4} Sodium Azide 10 p.p.m.P	Control 10 p.p.m.P
Transpiration-g.	4.8	9.7
Dry weight- mg.		
Root	105	125
Shoot	144	170
Phosphorus content $\mu C10^{-3}$		
Root	63.2	321.3
Shoot	16.1	546.7
Concn. P in transpiration stream $\mu C10^{-3}/g.$	3.3	56.4
T.S.C.F.	0.5	8.8
Transport Index %	20.3	63.1

translocated to the shoot was thirtyfour. Thus it appears that whilst the rate of transpiration was reduced by respiratory inhibitors, the transport of phosphorus across the root was independent of the rate of transpiration and was dependent upon metabolic factors.

A review of the literature (see Introduction) shows also that whenever a close relationship between transpiration and translocation was observed, the plants were supplied with a high level of salt, or the plants were of high salt status, whereas when absorption and translocation was shown to be independent of the rate of transpiration, low levels of salt were supplied to the plants. Thus it is apparent that the results that have been presented are in accord not only with the generally held views on the relation between metabolism and ion absorption, but also with the many observations on the relation between transpiration and ion absorption. In particular it is to be noted that the results of Hylmo (1953) are compatible with the results of the present investigations although his interpretation of his results to establish the passive transfer of ions across the root is not accepted. More recently Hylmo (1955) has attempted to reinterpret

the findings of Brouwer (1954) according to this theory. Brouwer (1953)(1954) had concluded that ion absorption is an active process, but that a high suction tension in the xylem can enhance the absorption of both water and ions, a view compatible with that here advanced. Hylmo's interpolation of values from one experiment to another, and his rejection of Brouwer's evidence that the inhibition of ion uptake by dinitrophenol was unaccompanied by a concomitant inhibitor of water absorption, make Hylmo's conclusions appear speculative. The rejection of Hylmo's interpretation by Brouwer (1956) and Arisz (1956) therefore appears fully justified.

It appears that the concept of the passive movement of ions across the root appealed both to Hylmo (1953, 1955) and Epstein (1956a, 1956b), because it has lately been realised that the outer surface of the cytoplasm of cells does not present a high resistance barrier to the entry of ions and that ions can diffuse readily in the cytoplasm (Jacobson and Overstreet 1950, Lundegardh 1950, Robertson 1951, Hope 1953, Sutcliffe 1954, and Epstein 1955) - that is to say the concept of 'apparent free space'.

It is well recognised that the concept of 'apparent free space' is in no way in conflict with

the evidence for the existence of a high resistance barrier across which ions are actively transported to the vacuole of a single cell. The evidence here reviewed is regarded as evidence for the existence of a barrier, possibly the endodermis which fulfills a similar function in the intact root. This interpretation in no way disputes the possibility that considerable zones of the root cortex are not separated from the outer medium by a high resistance barrier.

It must be emphasised however that whereas the existence of the tonoplast is well established similar evidence regarding the functions of the endodermis is lacking.

The investigations concerning the movement of phosphorus and rubidium into the leaves of sunflower plants which had been individually shaded (Experiments 21 and 22) showed that the shading of a leaf reduced the amount of phosphorus and rubidium transported into that leaf.

It was hoped that by using a short treatment period of 4 hours the effect of a reduction in the

carbohydrate level of the shaded leaves, which would reduce the capacity of the leaves to act as 'sinks' for nutrients, would be small. Although transpiration measurements were not made on the individual leaves, it would be expected that the shading of an individual leaf to 0.50 daylight would result in a marked reduction in the amount of water transpired by that leaf.

On the basis that the primary effect of shading was to reduce transpiration, it does appear that the transport of phosphorus and rubidium into individual leaves was to a certain extent controlled by the rate of transpiration of the individual leaves. Although this interpretation accords with the postulate that the transpiration stream passively transports ions in the xylem, further detailed investigations need to be carried out before it will be possible to reach any conclusions concerning the nature of the transport of ions into individual leaves.

The preliminary investigation on the effects of prolonged shading on the relationship between transpiration and translocation, described in Part II,

provides further evidence of a relationship between metabolism and the transport of phosphorus and rubidium across the root.

It was shown in Experiment 23 that the amount of phosphorus translocated was increased by a high rate of transpiration only in the plants pretreated in full daylight. However, transpiration had a much smaller effect on the translocation of phosphorus to the shoots than in the experiments described in Part I where similar concentrations of phosphorus had been employed. In Experiment 24 it was shown that the translocation of rubidium was not increased by a high rate of transpiration during the treatment period. It appears therefore, that transpiration was not the rate limiting process in translocation.

In the experiments described in Part I, the plants were transferred from a greenhouse to the controlled environment chambers immediately before the experimental treatment period. The light intensity, the humidity and the temperature differed markedly from those in the greenhouse. Thus, even when the plants were subjected to the highest light intensity in the controlled environment chamber and the concentration of the nutrient in the external medium was not altered, the plants were subjected to

considerable environmental changes. In the experiments described in Part II, however the plants which received the same light intensity in the pretreatment and treatment periods were subjected to no such change. It seems possible that the much smaller effect of transpiration on salt uptake shown in Part II than in Part I, at the same concentration of nutrient, may be a consequence of this. There is clear evidence of/a morphological adaptation of the plants to the different light intensities in the long pretreatment period employed in the experiments described in Part II. Equally there is evidence of/a physiological adaptation since the plants pretreated in the different light intensities showed differences in nutrient absorption when subjected to the same light intensity in the treatment period. In particular it is of interest in the phosphorus experiment (Experiment 23) where, although shading in the treatment period reduced the absorption of the plants which were subjected to full daylight in the pretreatment period, no such effect was observed in the plants that were shaded during the pretreatment period. These results are compatible with a view that a 'balance' had been attained between the metabolic processes responsible for active transport

of ions across the root and the utilisation in the shoot, with the result that variations in the rate of transpiration could exert but a small effect on the overall process of upward movement. It is to be emphasised that this suggestion rests on but two preliminary experiments. However, it is of interest that all available data, which suggests that transpiration has a large effect on salt uptake, appears to be derived from experiments in which the plants were subjected to considerable environmental changes. The possibility that these large effects are not representative of those occurring under natural conditions, therefore merits consideration. It is postulated that under natural conditions any effects due to changes in transpiration induced by shading may be of minor importance, for example in modifying the nutrient content of individual leaves (Experiments 21 and 22); metabolic factors appear to be dominant in determining the distribution of nutrients throughout the plant.

V. SUMMARY

Part I.

1. The relationship between transpiration and the absorption and distribution of phosphorus and rubidium by barley and sunflower plants was studied. Water culture methods were employed using ^{32}P and ^{86}Rb as tracers. The experiments were carried out in controlled environment chambers and the rate of transpiration was varied by shading.

2. It was found that by employing short experimental periods (24 hours) the effects induced by shading on salt uptake could validly be interpreted in terms of the effect of shading on transpiration.

3. When the concentration of phosphorus supplied to the plants was high (31 p.p.m. P) the absorption and particularly the translocation of phosphorus was markedly increased by a high rate of transpiration: when the concentration supplied was low (0.1 p.p.m. P) absorption was either independent or

was only slightly affected by transpiration. The concentration of phosphorus in the transpiration stream was calculated from the quantity of phosphorus translocated to the shoot and the amount of water transpired. By a comparison of the concentration of phosphorus on the external medium with the concentration in the transpiration stream, it was shown that rates of water and phosphorus movement through the plant were different. When the plants were supplied with the low level of phosphorus, the concentration of phosphorus in the transpiration stream exceeded that in the external medium to a highly significant extent. A measure of the extent to which the internal and external concentration differed was obtained by calculating the value 'T.S.C.F.' (i.e. $\frac{\text{Concn. Ion in transpiration stream}}{\text{Concn. Ion in external medium}}$) When the transpiration rate was low, T.S.C.F's greater than 100 were observed. In the plants supplied with the high level of phosphorus the T.S.C.F's approached unity.

4. From a study of the effect of transpiration on the absorption and distribution of phosphorus by

plants of different phosphate status, it was shown that it was only in the plants which possessed the greatest capacity for translocation that the amount of phosphorus translocated bore a close relation to transpiration. When the high level of phosphorus was supplied to the plants the effect of transpiration in increasing the translocation of phosphorus was greater in plants of high, than of low phosphate status.

5. The absorption and distribution of rubidium supplied at both a high and a relatively low level was influenced by the rate of transpiration. However, transpiration had a greater effect on absorption and translocation when the level of rubidium supplied to the plants was high. The T.S.C.F's were inversely related to the external level of rubidium and to the rate of transpiration.

6. Further evidence of an inverse relationship between the T.S.C.F's and transpiration was provided by two experiment in which plants transpiring at six different rates were obtained by the combinations of shading and of changing the osmotic concentration

of the external medium. Over the whole range the T.S.C.F. was reduced as the transpiration rate was increased.

7. The movement of phosphorus and rubidium into the leaves of sunflower plants which had been individually shaded, was studied. It was shown that the shading of a leaf reduced the amount of phosphorus and rubidium transported into that leaf.

Part II

8. Consideration was given to the effects of transpiration on uptake which occur when plants are grown continuously under different levels of light intensity. The plants were grown in the open or under shades throughout their whole life. It was shown that under these 'natural' conditions, in which the plants were grown without any environmental changes immediately before the treatment period, that the effects of transpiration on salt uptake were of minor importance. When plants were transferred from one light intensity to another before the treatment

period it was shown that it was only in the plants grown in full daylight that the rate of transpiration increased the translocation of phosphorus to the shoot. No such effect occurred with rubidium.

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