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Accumulation and distribution of phosphorus in pine seedlings (Pinus silvestris L.)

By

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Introduction

A large number of experiments have previously been made in the study of the uptake of water and minerals by roots and also how translocation occurs and where in the plant the accumulation of the substances which have been absorbed takes place. During recent years a number of excellent reviews on the subject have been published, *i.a.* BIDDULPH (1955), EPSTEIN (1956) and BOLLARD (1960). For practical reasons the majority of experiments have been carried out with test materials which have a swift cycle of development, such as beans, peas and cereals. But only comparatively few similar experiments have been carried out with slow-growing coniferous seedlings and in the cases when the mineral uptake here has been studied it has usually been in connection with mycorrhiza experiments. In this respect it has emerged *i.a.* that the mycorrhiza fungi comprise an important complement to the host plant's root system. The host plant is thus supplied with phosphates and other minerals through the mycelium (MELIN & NILSSON, 1958). KRAMER & WILBUR (1949) have further demonstrated that root sections with mycorrhiza possess a large capacity for accumulating radioactive phosphate.

The following paper is a study of the mineral uptake of pine seedling (*Pinus silvestris* L.) roots without mycorrhiza. This is a field which up until now has been but briefly dealt with. In the experiments involved both quick-growing roots and older partly suberized root systems have been used.

Material and method

The test material consisted of pine seedlings (*Pinus silvestris* L.) of varying ages (7 days to 6 months). In the cases when germinal shoots were used these were reared with continuous aeration in a water culture with a 20 % concentration of Shive's nutrient solution. The solution was changed once a week. When the seedlings involved were more than one month old they were reared under sterilized conditions in Erlenmeyer flasks (1000 ml) with terralite and nutrient solution of the same concentration as above. There was very little evaporation from the flasks and consequently it was only necessary to add to the solution about every third month. Seedlings in terralite were used in preference to ones in water cultures since the former developed more forcibly and apparently more naturally. The photoperiod in both cases was 15 hours, the light source (Phillip's fluorescent tubes 20 W/33 or 65 W/33) gave about 4000 lux. The mean 24 hour temperature was 23 ± 3°C.

The P^{32} phosphorus isotope which was used in the experiment was bound in the form of orthophosphate and came from the Radiochemical Centre, Amersham, England. Varying quantities of radioactive phosphate (0.25—4.00 millicurie/litre), depending upon the size of the root units which were treated in the isotopic nutrient solution, were added to the full concentration of Shive's nutrient solution, the latter being subjected to intense aeration for a period of 18 hours. The entire root system, or parts of it, were thus able to absorb the radioactive nutrient solution for a period of six hours (25°C, 4000 lux), whereupon it was rinsed five times in Shive's nutrient solution at full concentration and then dried out for 18 hours at 95°C.

The procedure continued as follows:

a. In order to provide an autoradiogram the radioactive seedlings were pressed against an X-ray plate in a darkened room for a period of from 18—48 hours (film: Gevaert Osray, exposer: Gevaert 209A, fixing solution: Gevaert 333) and/or

b. The seedlings were cut up into suitable sections and the quantity of P^{32} was established with the assistance of a Geiger-Müller apparatus (Tracerlab's Autoscaler SC-51 with Tracerlab's Geiger tube TGG-2).

A test revealed that there was no difference in the measured result between a dried sample and an ashed sample of the same material. This was due to the fact that the comparitively intense Beta rays were only slightly absorbed by the tissues. Therefore the radiation intensity of the dried material was measured in all the tests except one where, for practical reasons, it was necessary to ash the material in order to reduce its volume.

For the purpose of investigating the absorption capacity of radioactive phosphate in the different zones of an unramified root an absorption cell similar to the one described by WIEBE & KRAMER (1954) was used. As can be seen from Fig. 1 this cell consists of an inner and an outer fluid system. The inner system comprises a glass tube joined by a plastic tube (inner and outer diameters 5 mm. and 7 mm.) with two holes through which the root was passed. Water-free lanolin was found to be the most suitable luting material for closing up the hole around the root. In the inner system a fixed 5 mm. segment of the root was treated with a radioactive solution which flowed slowly passed the root. The remainder of the root was immersed in a nutrient solution that had no addition of P^{32}. The absorption cells were easily coupled in series.

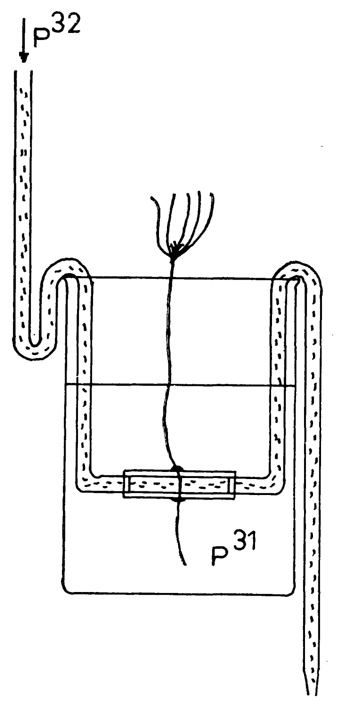

Fig. 1. Absorption cell (inner fluid system) for investigating the P^{32} absorption in a 5 mm. zone of the root. The remainder of the root section only came in contact with non-radioactive solution in the outer fluid system.

Guidance experiments with different test conditions.

Knowing the P^{32} content of the shoot and root it was possible to determine *i.a.* the percentual amounts of P^{32} uptake. It is important to bear in mind meanwhile that these values only apply to those conditions which prevailed immediately after the six-hour treatment in the radioactive solution, and not to the final distribution which was related to the new conditions which arise, for example, when the free phosphate ions which do not accumulate permanently in the root cells, are enabled to be translocated to the shoot.

The following test represents an example. The entire root systems of intact plants were treated for six hours in isotopic nutrient solution, after which the roots were rinsed. A quarter of the material was dried and the amount of P^{32} in the roots and shoots was determined. The remaining plants were placed in a nutrient solution without the addition of radioactive phosphate. After another 30, 78 and 126 hours the same procedure was repeated, each time with a quarter of the material. The results are shown in the graph in Fig. 2. From this it emerges that 18 % of the phosphate uptake was to be found in the shoot shortly after treatment in the isotopic solution. When the material was placed in nutrient solution containing no P^{32} a further quantity of the P^{32} which had been absorbed by the root was translocated to the shoot. After about five days a state of equilibrium was reached, whereupon some 50 % of the phosphate uptake could be found in the shoot while the other 50 % remained in the root system. Similar observations were made by BIDDULPH *et alia* (1958) when they used beans as test material.

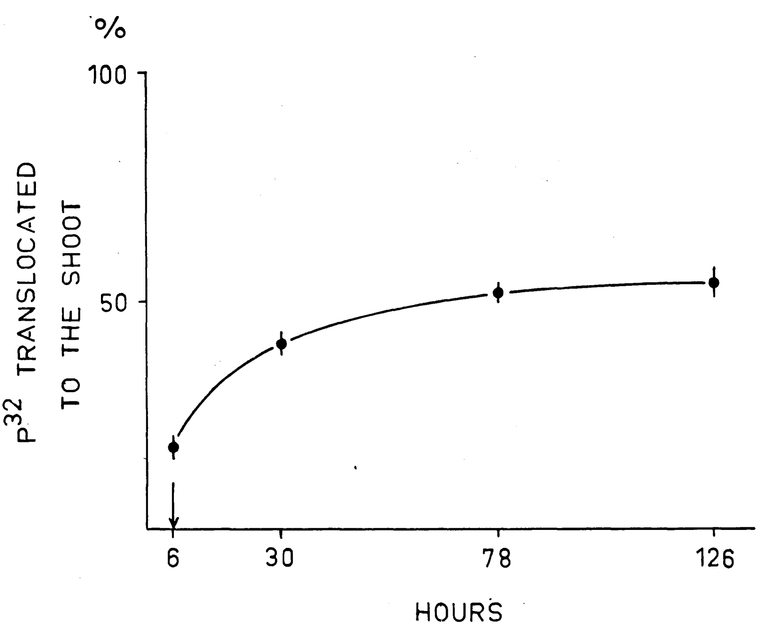

Fig. 2. The P^{32} translocated up to the shoot, as a percentage of the total quantity of absorbed radioactive phosphate, directly after six hours treatment in radioactive nutrient solution, after 24, 72 and 120 hourly treatment in P^{32}-free nutrient solution. The readings were carried out after the material had dried out for 18 hours at 95° C. The mean values represent five readings.
Plants: *Pinus silvéstris* L., age: 6 months.

In order to be able to make a more detailed investigation of the P^{32} uptake in the different root zones, by means of the absorption cell, it was found to be more suitable, for practical reasons, to select a material in which the secondary root development had not yet begun, i.e. seedlings less than 20 days old. The immediately subsequent investigations revealed, however, that parallel tests gave extremely varied results, a fact which has often been pointed out in the literature pertaining to work of a similar kind. Thus KRAMER & WIEBE (1952) found remarkable irregularities in the accumulation of P^{32} in barley roots. Autoradiograms of *Pinus taeda* roots revealed in some cases a large accumulation of radioactive phosphate in the actual root tips — the most common state — in others, on the other hand, the accumulation was greatest one to several centimeters behind the root apex. Analogous observations were also made by KRAMER & WILBUR (1949) in investigations into the phosphate absorption of pine roots (*Pinus taeda* and *P. resinosa*) with and without mycorrhiza.

The next four points deal briefly with factors which to a greater or lesser degree could be expected to affect the test results for the accumulation and distribution of radioactive phosphate. By bearing these factors in mind it is possible to decrease the irregularities considerably.

1. *Aeration of the solution.* As was earlier pointed out in the method discription there was no aeration of the isotopic solution during the period of treatment; the radioactive phosphate was simply added to a well-aerated nutrient solution. A test revealed that the absorption of the radioactive phosphate ion did not increase with continued aeration. HYLMÖ (1953) came to the same conclusions in his work on the calcium and chloride uptake of peas. Neither from the practical viewpoint was it suitable to carry out aeration during treatment, since the difficulty was bound to arise of avoiding splashing the shoot with the isotopic solution.

2. *Correlation between transpiration and ion uptake.* There have been detailed discussions of the importance of transpiration in relation to ion uptake (*i.a.* FISCHER, 1958, SCOTT RUSSEL & BARBER, 1960). One research group (HYLMÖ, 1958, EPSTEIN, 1956, *et al.*) considers that the ions move passively with the transpiration current from the root surface into the xylem, while another group (BROYER & HOAGLAND, 1943, BROUWER, 1956 *et al.*) maintains that the ion uptake is an active process, depending for its energy upon the root metabolism and that in this respect the magnitude of the transpiration is of no significance. In connection with this it might be mentioned that earlier literature (SKOOG *et al.*, 1938) supported the hypothesis that there can exist a certain 24-hour variation in the root's symplast capacity to absorb and transfer ions from the epidermis to the xylem tissues. In view of this, according to SKOOG *et al.*, short interval tests ought to be carried out at specific periods throughout the course of the 24-hour stretch.

In an investigation into the uptake and translocation of radioactive phosphate in beans HANSON & BIDDULPH (1953) found *i.a.* a significant difference in the quantity of radioactive phosphate which was translocated to the shoot between 10am—2pm and 2pm—6pm under identical circumstances. According to them the reason for this difference was due to the fact that the roots had varying access to carbohydrates, which in its turn resulted in variations in P^{32} absorption. Comparative investigations into the ion uptake and transportation should thus be made at the same time of the day or night and also under corresponding light, temperature and humidity conditions, since it is clear that the extent of ion translocation from the root to the shoot depends upon the magnitude of the transpiration.

3. *Age of the plants.* The pine seedling's primary root in the nutrient solution grew quickly, but then, after not more than 20 days, a marked stagnation was observable in the longitudinal growth. This indicated a reduction of activity in the primary root. Since it had been made clear during previous investigations (HOAGLAND, 1944, WIKBERG, 1956, *et al.*) that the ion accumulation is correlated with the existing metabolic activity, it can be assumed

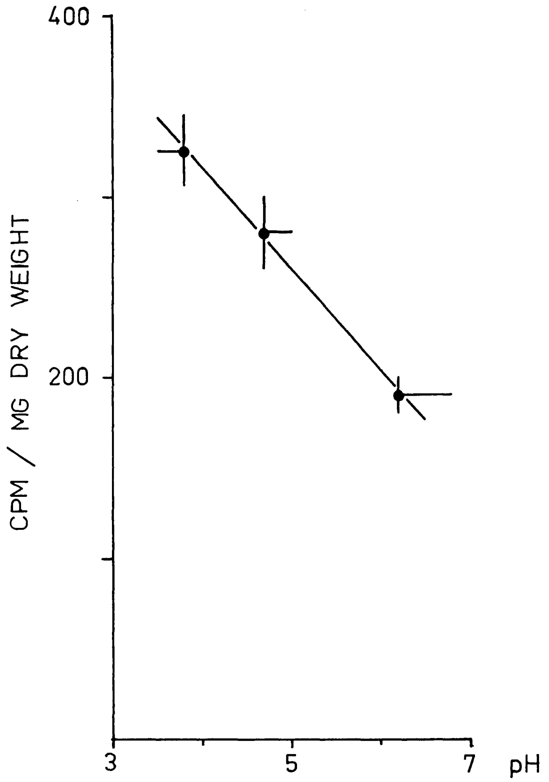

Fig. 3. Connection between the quantity of P^{32} in shoot / mg. dry weight and pH in the nutrient solution. — After an acclimatization period of 3 days in solutions of varying degrees of acidity the radioactive phosphate was added to the solutions. Readings were taken after the seedlings had been treated for 6 hours in the radioactive solution and dried out for 18 hours at 95° C. The mean values represent five readings. The mean errors and changes in pH during the progress of the experiment are shown in the figure.
Plants: *Pinus silvestris* L., age: 12—15 days.

that there are variations in the P^{32} accumulation of the primary roots in plants of different ages. This is further confirmed by another test which will be mentioned later in this paper. Comparative tests should thus be carried out with plants of similar age.

4. *The pH of the solution.* In one of KRAMER's papers (1951, table 1, page 32) it is pointed out that the root apices of the *Pinus taeda* accumulated four times as much phosphate at pH 4.7 than at pH 5.7. In order to establish to what extent the translocation of phosphate to *Pinus silvestris* shoots is affected by the solution's pH the following test was carried out. 12 day old seedlings were transferred to Shive's nutrient solution at full concentration. The solution was adjusted to the required pH value with NaOH and HCl. The variation area investigated was pH 3.5—6.8. After acclimatization periods of 1, 2 and 3 days respectively the radioactive phosphate was added. The seedlings were treated for six hours whereupon the dry weight and the radioactivity of the shoot was determined. The test showed that more phosphate was transported to the shoot in the more acid solutions. The result, as shown in Fig. 3, indicates that the experiments should thus be made with solutions of equal acidity.

Results and discussion

Accumulation of phosphate.

A. Accumulation in root sections of different ages.

As pointed out on page 9 the investigations of HOAGLAND *et al.* have shown that ion accumulation and metabolic activity are two correlated factors.

The longitudinal growth stagnation which was observable in the primary root of a pine seedling after some 20 days, indicated a reduced metabolic activity in the root. It seems probably therefore that one and the same root zone, counted from the root apex, can accumulate different quantities of ions from the surrounding nutrient solution, all depending upon which state of growth the root is in at the time when the test is made. The experiment below confirms that this is the case.

In this experiment isotopic nutrient solution was applied to intact seedlings of varying ages. After 6 hours treatment the roots were cut up into 5 mm. lengths (0—5, 5—10 etc.) starting from the root apex. The amount of P^{32} in CPM (counts per minute) contained in each zone was established. Hereby it was found preferable to set the root zone's radioactive phosphate content in relation to a fixed length unit instead, for example, of the dry weight. This was because the content was so little (approx. 1.0 mg./5 mm. zone) that accurate measurements were difficult to establish. The test material had been selected in such a way that if, for example, an 0—25 mm. segment was being examined seedlings with not less than 25 mm. and not more than 29 mm. were taken. The apical 0—5 mm. zone comprised partly the root apex, the meristematic region and the undifferentiated tissue and partly the cells in process of longitudinal growth and differentiation. In the two subsequent zones (5—10 and 10—15) the development of the tissues had advanced and the protoxylem which had formed emerged in the shape of a "Y" — a charateristic aspect of the *Pinus* species (LAING, 1932). Nearer the base of the root the cortex was made up of several layers of cells and it was also possible to establish the presence of an endodermis. The construction was about the same as the ADDOM's discription (1950) of the swift growth of the long roots in 3-year old *Pinus taeda* plants.

The results of the treatment with radioactive phosphate are shown in Fig. 4 where mean values and mean errors for five readings per zone and age group are shown. Test readings with 7-day old seedlings show relatively large quantities of P^{32} in all three of the existing root zones, while in the case of 11-day old seedlings an entirely different picture was revealed. In the latter case the difference in the quantity of P^{32} between the meristematic zone and the four other zones was evident, but in 18-day old seedlings this difference had evened out and the quantity of radioactive phosphate was comparatively low in all the zones.

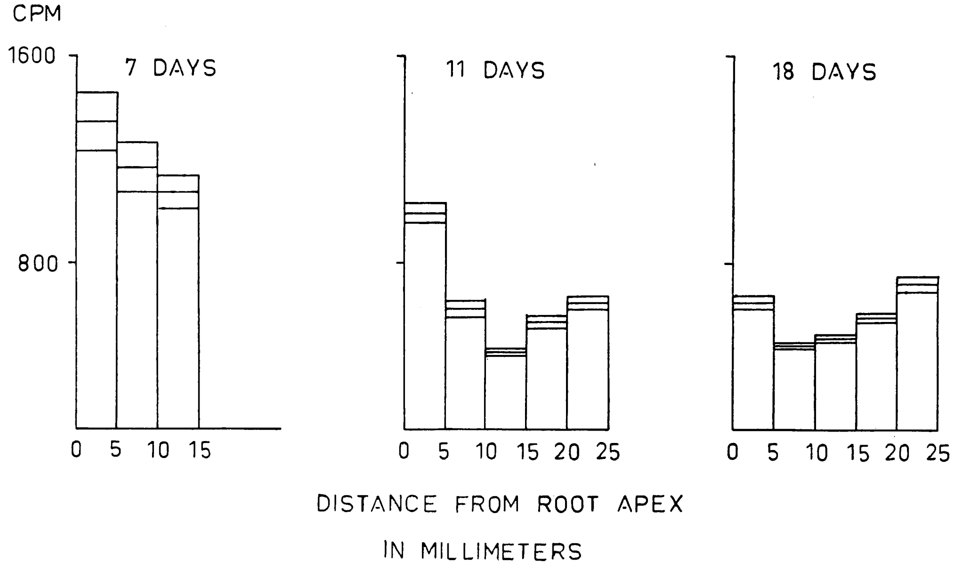

Fig. 4. The distribution in different 5 mm. root zones of radioactive phosphate — reckoned in CPM — which is absorbed by the root during a period of 6 hours in 7, 11 and 18-day old intact seedlings. The readings were made after the material had been dried out for 18 hours at 95° C. The mean values and mean errors represent five readings. Plants: *Pinus silvestris* L.

The measured quantity of P^{32} per zone constituted not only the accumulated quantity of radioactive phosphate in the root cells, but also of free phosphate ions which was available for translocation to other parts of the plant. This, however, does not affect the conclusion that can be drawn from the experiment, *i.e.* that the accumulation capacity of the different root zones and particularly the meristematic 0—5 mm. zone changes with the growth of the root. In other words it is impossible to generalize on a result which is arrived at concerning the phosphate accumulation in different root segments of, for example, 15-day old seedlings, and apply this to the entire growth of the root. The result shows only the conditions which exist at a certain age in the growing cycle.

B. Accumulation and oxygen consumption.

Machlis claimed (1944) that the oxygen uptake was highest near the barley root apex. Berry & Brock (1956) and Norris (1951, 1956) made similar observations using onion roots as test material. This condition is explaned by the fact that the plasma content is greatest in the apical parts of the root.

Prevot & Steward (1936) and Steward *et al.* (1942) found through investigations of the ion accumulation of corn roots that in comparison with the other parts of the roots the apices accumulated considerably more Br^-, Rb^+ and K^+ from the surrounding solution. Kramer and Wiebe (1952) made similar observations concerning the phosphate accumulation of *Pinus resinosa* roots. The latter also found that P^{32} uptake was reduced to less than 10 % of the control through the effects of respiration inhibitors or

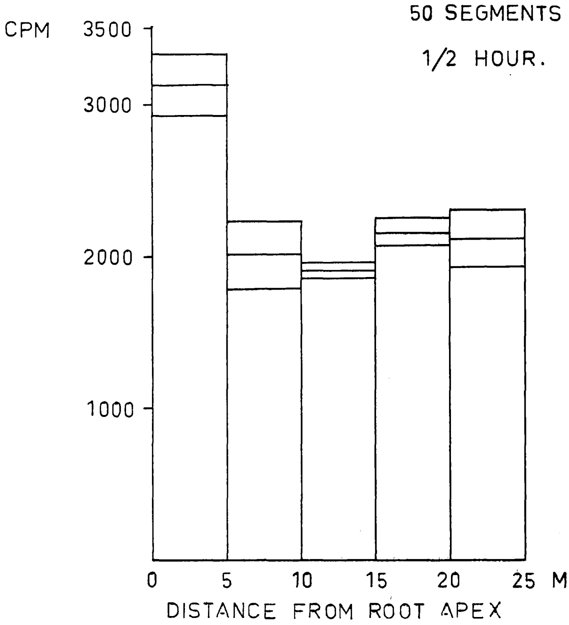

Fig. 5. Quantity of radioactive phosphate absorption — estimated in CPM — during six hours in detached segments from different root zones. Readings were made when, after treatment with radioactive solution, the material had been dried out for 18 hours of 95° C. The mean values and mean errors represent five readings.
Plants: *Pinus silvestris* L., age 13 days.

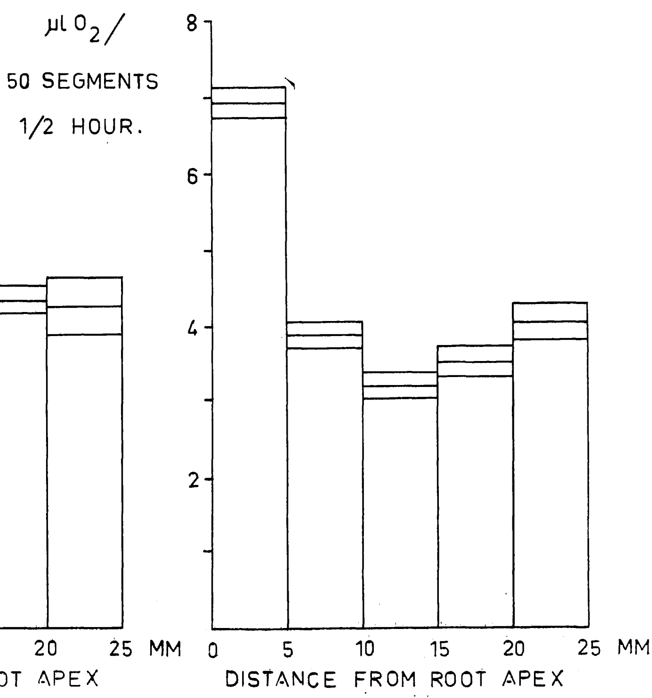

Fig. 6. Oxygen consumption in detached segments from different root zones, estimated in microliter per 50 segments and 30 minutes. The mean values and mean errors represent sixteen readings.
Plants: *Pinus silvestris* L., age 13 days.

low temperatures. These results indicate a prevailing correlation between respiration and ion accumulation.

In order to examine the conditions in the primary roots of pine seedlings the following experiment was carried out with 13-day old seedlings with 25—29 mm. long roots.

a. The roots were cut up into 5 mm. segments and immersed in an isotopic solution for 6 hours. Afterwards the root segments were rinsed 5 times in a P^{32}-free solution and then the amount of P^{32} in CPM in each segment was determined. Fig. 5 gives mean values and mean errors for five readings per root zone.

b. The roots were cut up into 5 mm. segments. The oxygen consumption was measured in a Warburg apparatus for two series of 50 segments per zone and 30 minutes (Fig. 6).

Figs. 5 and 6 show that the meristematic 0—5 mm. zone had, in comparison with the other parts of the root, the largest oxygen consumption and also absorbed the largest quantity of radioactive phosphate. It is interesting to note the similarity between the distribution of P^{32} in the different root zones in this experiment and the earlier one with intact seedlings of practically the same age (Fig. 4).

The experiment thus confirms the earlier result (see above) and shows that the phosphate accumulation in the different root zones is correlated with the oxygen consumption and metabolic activity of the segments.

Distribution of phosphate.

A. Distribution from different root zones.

The above mentioned experiments on respiration and P^{32} accumulation in detached segments from different parts of pine roots laid the ground for further experiments in this field. The object was *i.a.* to try to establish from which parts of the roots of intact pine seedlings the translocation to the shoot mainly occurs. Experiments which have been carried out by WIEBE & KRAMER (1954) and CANNING & KRAMER (1958) have shown that a comparitively small part of the ion uptake in the meristematic zones of corn seedlings is available for translocation to the shoot. WIEBE & KRAMER found that the large amount of upward translocation took place from a zone 30 mm. behind the root apex.

The material in the experiment below consisted of 11—15 day old *Pinus silvestris* seedlings with 25—29 mm. long roots. The experiment was carried out with the assistance of the previously described absorption cell (page 6). After treatment in an isotopic solution of the 5 mm. zones 0—5, 5—10, 10—15, 15—20 and 20—25 mm. from the root apex the material was cut up into parts which were checked as follows:

a. The shoot and the part of the root above that treated with P^{32}.
b. P^{32} treated 5 mm. zone plus 1 mm. of the root at either end (= the plastic tube's outer diameter in the absorption cell).
c. The part of the root below the zone treated with P^{32}.

The results are shown in Fig. 7. The autoradiograms concurred with the CPM values.

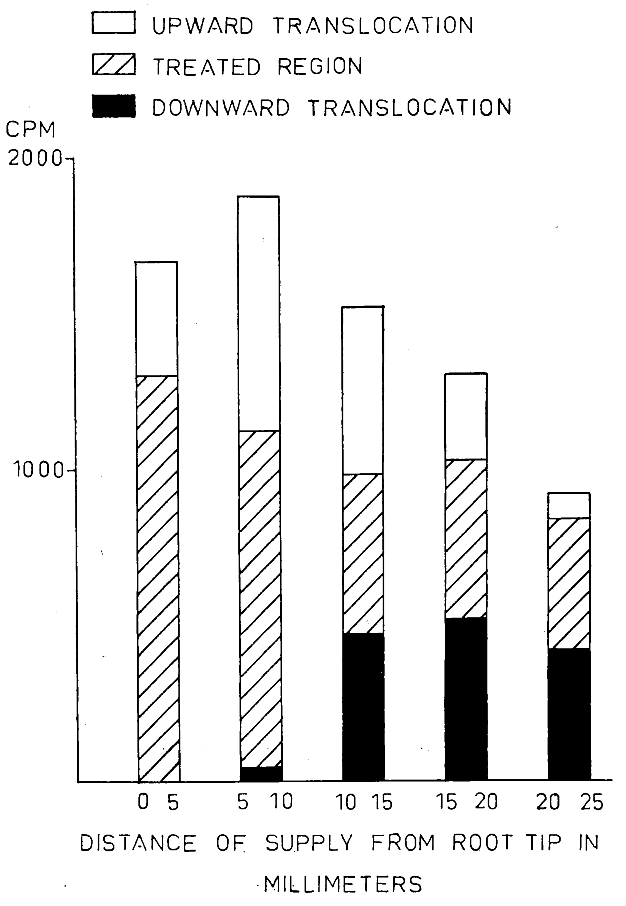

Fig. 7. Distribution of radioactive phosphate — estimated in CPM — absorbed during six hours in different 5 mm. root zones by intact seedlings. The experiment was made in absorption cells (cf. Fig. 1). The readings were taken when the material, after treatment in a radioactive solution, and been dried out for 18 hours at 95° C. The mean values represent five readings.
Plants: *Pinus silvestris* L., age: 11—15 days.

The largest phosphate uptake was in the 5—10 mm. zone and it was somewhat lower in the apical zone, although the difference was not significant. The absorption capacity lessened then towards the root base. The up-words translocation was greatest in the zones nearest the apical zone and less in the basal parts. On the other hand 40—50 % of the phosphate uptake was translocated downwards from the basal zones. The conditions between the accumulated quantities of radioactive phosphate in the different zones agreed with those of earlier experiments (cf. Fig. 5), with the exception of the 5—10 mm. zone which proportionately accumulated a larger quantity of phosphate.

A comparison with the results achieved by WIEBE & KRAMER (1954) concerning the uptake and translocation of P^{32} in corn plants reveals that similarities exist between the two materials. There are a couple of departures, however. The upward translocation from the apical root zone of corn plants was about 2 % of the total uptake of P^{32}, while the corresponding value for pine seedlings was about 20 %. This difference probably depends upon the fact that WIEBE & KRAMER worked with 3 mm. segments and that the protoxylem was first encountered 6—9 mm. from the root apex in the corn seedlings. Since the translocation of water and its dissolved substances from root to shoot takes place through the xylem, in their case the primary condition for the transfer of ions from the apical 3 mm. segment to other parts of the plant was absent. In quick-growing pine roots on the other hand the first protoxylem builds up only 3—5 mm. from the apex. This is probably the reason why a relatively large amount of phosphate was translocated upward from the 0—5 mm. zone in the pine seedlings.

WIEBE & KRAMER also observed that both the P^{32} and S^{35} was translocated right down to the root apex from the root's basal parts, while the I^{131} and the checked cations did not get farther than 3—15 mm. from the root point. This last condition is also true of P^{32} in pine roots. The radioactive phosphate which was conveied to the basal parts of the root was not on any occasion translocated downwards to the meristematic complex, but remained chiefly in the treated zone and got no farther than to the 5—15 mm. segment.

As has already been mentioned above the phosphate which had been absorbed by the oldest root zone (20—25 mm. zone) and had not accumulated there was transported mainly downwards. Several tests revealed that 40—50 % of the total radioactive phosphate uptake was transferred from the 20—25 mm. zone to the younger parts of the root while 10—25 % of the phosphate was transported to the shoot. WIEBE & KRAMER (1954) found the same tendency, but did not comment upon it. A simple study of the values that WIEBE & KRAMER give (table 1, page 345) shows that in their experiment 27 % of the phosphate was transported downwards from the basal parts of the corn root. It should be noted here that the phosphate which remained in the area 2 cm. below the treated section is not included here.

The fact that the concentration decreased in the 15—20 mm. zone and then increased again in the 5—15 mm. segment indicates that the downward translocation was to a great extent of an active nature rather than a diffusion process. Another point in support of this interpretation is the result which derived from an experiment where the shoots were detached from the roots before the 20—25 mm. zone was treated with an isotopic solution in an absorption cell. The roots in their entirety were placed under the outer fluid layer. In this case only 8 % of the radioactive phosphate absorption was carried downwards and this for the greater part did not get beyond the 15—20 mm. zone. The same result was achieved when the glycose (0.5 %) was added to the outer solution in the absorption cell in order to compensate for the absence of carbohydrates from the shoots. The last experiment shows that since the shoot was detached from the root the possibilities of active translocation were eliminated and that the translocation of phosphate in this case was apparantly a result of diffusion.

B. The effect on distribution of root decapitation.

In order to study the effects of root decapitation on distribution a series of tests were made in which 5 mm. of the apical root zone was cut off. These investigations are of interest insomuch that a new method is now being put into practice whereby young pine and fir transplantation is being replaced by thinning and root pruning. In connection with this it can be mentioned that Björkman (1953) carried out experiments whereby he showed that a certain amount of root pruning in young fir plants can be more to their benefit than injury. This is probably because root pruning can lead to an increase in the process of root growth.

Distribution from the decapitated root to the shoot

Earlier experiments have shown that only 10—20 % of the phosphate absorbed by intact roots during a treatment time of six hours can be traced to the shoot, when a phosphate reading is made directly after the treatment. The next step was thus to establish whether or not the decapitation of the apical zone of the root resulted in any difference in the amount of phosphate which was transported to the shoot.

A number of tests in this field revealed that much more radioactive phosphate was conveyed to the shoot and the distribution of phosphate uptake between the shoot and the root was affected if the apical zone was removed. This applied both in the case of seedlings (Table I) and older plants where the main root was decapitated. This effect was accentuated even more if the 0—5 mm. zones of the side roots were also decapitated. In the same way when only the lowest 5 mm. zones of the intact and decapitated roots were treated with an isotopic solution a similar tendency was observable. It was evident that the extra supply of phosphate that reached the plant as a result of the decapitation was absorbed through the cut surface. The reason for this would seem to be that through the cutting process the barrier formed by different cell tissues between the outer solution and the xylem was removed. Once the cut had been made the phosphate in the surrounding solution came into direct contact with the xylem and was more easily conveyed up to the shoot by the transpiration current. BROYER & HOAGLAND (1943) made a similar observation in their tests. When the roots of intact corn plants were injured through being immersed in a hypertonic common salt solution they became super-absorbent.

The question is, however, whether or not root decapitation also leads to a larger amount of solution being conveyed to the shoot as a result of increased transpiration, since the plants both before and during the treatment in radioactive solution had optimal access to water. In order to investigate this tests were made with two different relative humidity conditions. In one case the plants were treated in the existing humidity of the laboratory and in the other case in a chambre with almost saturated humidity. It thus emerged that a decrease in the relative humidity resulted in a comparatively larger translocation of P^{32} to the shoot in plants with decapitated roots than in plants with an intact root system (Table I). This indicates that decapitation also results in increased transpiration.

	Relative humidity	CPM		% in shoot
		Total uptake	in shoot	
Plants with intact root systems ...	low	3 523	470	13
	high	2 966	104	3
Plants with decapitated 5 mm. zones	low	11 334	8 740	77
	high	3 175	1 103	34

Table I. Total uptake of radioactive phosphate reckoned in CPM in plants with intact and decapitated roots at low and high relative humidity. Also the distribution to the shoot. The mean values represent five readings. Material: 15 day old equally-sized *Pinus silvestris* seedlings.

This decapitation effect, however, was a temporary one which scarcely lasted more than 24 hours. It resulted from a test where half of the seedling roots were decapitated, whereupon a third of these were treated immediately in an isotopic solution, another third after 24 hours and the remainder after 48 hours (Fig. 8). In the case of the immediately treated material, as earlier, considerably larger amounts of phosphate were transported to the shoot in the decapitated plants, but after 48 hours there was no difference between the decapitated plants and the intact plants, presumably on account of the fact that the cut had healed.

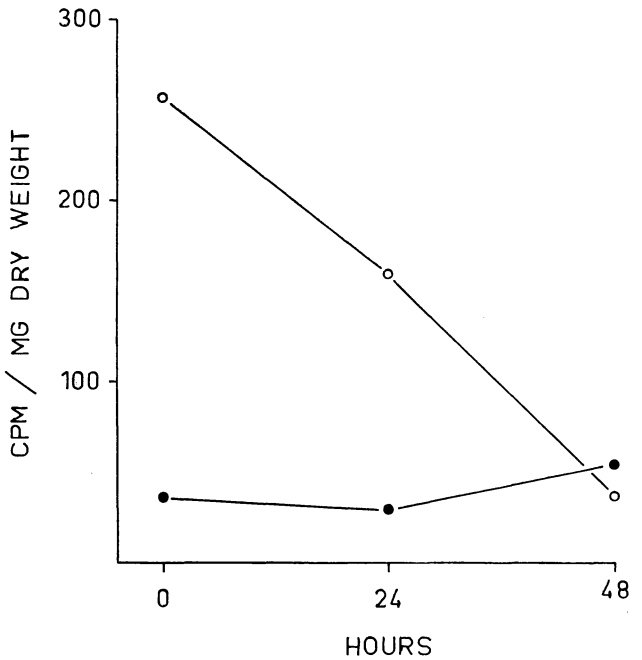

Fig. 8. Quantity of P^{32}, measured in CPM per mg. shoot dry weight, transported to the shoots of plants with the 0—5 mm. root zone decapitated (open rings) and of intact seedlings (spots). Roots were treated immediately, 24 hrs and 48 hrs after decapitation. The mean values represent five readings.
Plants: *Pinus silvestris* L., age: 15 days.

Distribution from the basal parts in a decapitated root

As has been stated earlier the downward translocation of P^{32} from the basal parts was unexpectedly large (page 16). The test below was thus carried out in order to establish whether the decapitation of the root's 0—5 mm. zone affects the distribution of the phosphate absorbed in the basal parts of the root. Such an effect would seem to be probable since decapitation takes away that part of the root which has most respiration (Fig. 6) and consequently the greatest carbohydrate consumption.

In the experiment the following 20—25 mm. zones were treated with a radioactive solution in an absorption cell:

a. from intact plants,
b. from plants with a decapitated 0—5 mm. zone,
c. from plants with a decapitated 0—5 mm. zone where the entire cut surface had been sealed with lanolin. (It was revealed that radioactive phosphate cannot be absorbed through a lanolin-sealed cut surface.)

The results are shown in Table II.

	% of total P^{32} uptake in:		
Treatment:	shoot	treated root zone	root part below treated zone
a) intact plants	21 ± 2	38 ± 3	41 ± 4
b) 0—5 mm. zone severed	47 ± 3	38 ± 4	15 ± 1
c) 0—5 mm. zone severed and cut surface sealed	30 ± 4	41 ± 3	29 ± 5

Table II. Percentual distribution of radioactive phosphate uptake during 6 hours in the root zone 20—25 mm. behind the root apex. The mean values represent five readings. Material: 15-day old *Pinus silvestris* L. seedlings.

In the intact plants the largest quantity of phosphate which was absorbed by the 20—25 mm. zone and not accumulated was transported down to the root apex (Table IIa). After decapitation approx. 45 % was transported to the shoot and only 15 % to the root apex (Table IIb). After severing and sealing intermediary values resulted (Table IIc). Decapitation of the apical zone affected neither the basal zone's accumulation capacity nor its absorption capacity. This concurs with what has previously emerged, i.e. that the greater part of the phosphate which comes to the plant through decapitation is absorbed through the cut surface (page 18).

The variations in P^{32} distribution from the 20—25 mm. zone, following decapitation of the apical zone, may have been caused by increased transpiration through the cut surface or, in other words, increased rate of flow in the xylem. This could mean that larger quantities of P^{32} were carried away from the basal root zone and transported to the shoot. In the case where the cut surface was sealed with lanolin (Table IIc) the oppurtunity for increased rate of flow in the xylem was elminated since the open xylem was sealed off. Despite this the P^{32} distribution was still different from that in the intact material. This result can be interpreted to mean that severing also leads to disturbances in the translocation in the phloem. The experiment gave no further information on the problem.

As shown in the previous experiment the decapitation effects are of a temporary nature since they are eliminated by the healing of the cut and since other root meristems increase their activity.

C. Phosphate accumulation in the root collar.

In certain cases it was observed that phosphate collected in the root collar in pine plants. These accumulations never occur in very young seedlings, but appear first when the plants are at least two months old. The accumulations were irregular and of varying size. Figure 9 shows an autoradiogram of a 2-month old plant with a typical phosphate accumulation in the root collar. As a result of the accumulation it is probable that the supply of phosphate to the shoot was less. Since this symptom appeared irregulary there were variations in the distribution of the total quantity of P^{32} uptake in the plant.

WIEBE & KRAMER (1954) observed the same phenomenon in corn seedlings and claimed that the phosphate which accumulated in the root collar came from the transpiration current in the xylem. MORELAND's experiment (1950) in the translocation of radioactive phosphate in the *Pinus taeda* also showed that the phosphate is absorbed by the existing tissues around the xylem. There does not seem to be any grounds, however, for assuming that the area around the root collar accumulates more phosphate than the nearer parts of the stem and root.

The following experiment was carried out in order to examine this point more thoroughly. 14 three-month old plants were treated for 6 hours in an isotopic solution. After rinsing half of the material was immersed for 18 hours in a nutrient solution of the same concentration as the isotopic solution, but without the addition of P^{32}. One centimeter long sections were taken from the stem, the basal root region and the root as shown in Fig. 10 and these were checked in CPM.

Fig. 9. Autoradiogram of a two month-old seedling with an »accumulation» of radioactive phosphate in the root collar. Plant: *Pinus silvestris* L.

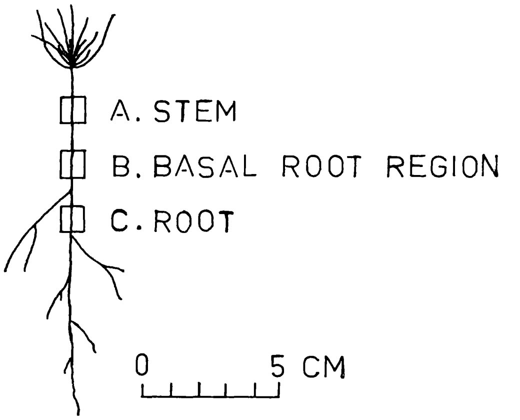

Fig. 10. Schematic sketch of a pine seedling with marked test zones. See text and Table III for further explanation.

The values which are shown in Table III indicate that it was not a question of a permanent accumulation, but rather of a temporary accumulation of radioactive phosphate in the root collar, since the phosphate was carried away when the material was placed in an isotope-free solution. As has been pointed out before there were great variations in the quantities which collected in the root collar of the different individual plants. This is further reflected in the high mean error of the mean CPM value from root collar tests in materials which had not been subjected to any after-treatment.

Treatment / 1 cm. tests of	Relative CPM: 6 hrs in radioactive solution	Relative CPM: 6 hrs in radioactive + 18 hrs in non-radioactive solution
Stem	68 ± 11	59 ± 7
Root collar	224 ± 65	91 ± 8
Root	100	100

Table III. Relative CPM from different segments — see Fig. 10 — following treatment of the root systems of three-month old plants in radioactive solution and after-treatment of half of the material in a solution without the addition of radioactive phosphate. In both cases readings were taken after the material had been dried out for 18 hours at 95° C. The values for the root segments have been made up against a ratio of 100. Mean values of seven readings.

Moreland (1950) found that radioactive phosphate is transported more quickly through the root than the stem in 11—12 year old *Pinus taeda* plants. The explanation he gave was that the tracheids of the roots were longer and had thinner walls, thus giving less resistance to the ions in the upward transport. Even if this difference also exists in *Pinus silvestris* tracheids the accumulation of radioactive phosphate in the basal root region cannot be solely attributed to this, since the accumulation, as has already been pointed out, is of an irregular nature. On the other hand possible morphological differences in the transfer from the radial to the colateral vascular bundles in the root collar may be the reason for this condition. This problem has not been closely investigated, however.

Summary

I. The investigation deals with the accumulation of radioactive phosphate in roots and the distribution from the roots, or parts of these, in pine seedlings (*Pinus silvestris* L.).
The seedlings were entirely without mycorrhiza.

II. The experiments were carried out:

a. under similar light, temperature and humidity conditions,
b. with constant pH in the nutrient solution with P^{32} content,
c. at the same time of the day or night,
d. in most cases with seedlings of fairly similar age.
By maintaining this system it was possible to reduce considerably the variations in the results.

III. Tests on phosphate accumulation showed:

a. that the accumulation capacity of different root zones varied pronouncedly with the roots growth,
b. that the accumulation in the different root zones was correlated with the zones' oxygen consumption.

IV. With the help of an absorption cell the P^{32} distribution from different 5 mm. zones of the roots of 11—15 day old seedlings was investigated.

The tests showed:

a. that the two root zones next to the apical zone absorbed the largest amount of the phosphate which was carried to the shoot,
b. that translocation from the basal parts of the root went chiefly downwards,
c. that the phosphate which was absorbed by the apical zone remained for the most part in that zone.

V. When the apical zone of the root was decapitated:

a. more phosphate was carried to the shoot, partly because the xylem was opened as a result of the cutting so that absorption was easier and partly because there was an increase in transpiration,
b. a larger quantity of the phosphate absorbed by the basal parts of the root was carried to the shoot, while translocation to the younger

root parts diminished. The absorption and accumulation capacity of the basal parts of the root was not affected by decapitation.

The decapitation effects were of a temporary nature and only lasted about 24 hours.

VI. When the older plants were treated in a nutrient solution with a P^{32} content there developed in the root collar a temporary accumulation of P^{32} which was both irregular and of varying quantity. This phenomenon could be the reason for variations in the distribution of P^{32} in the shoot and the root.

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