

## SHORT COMMUNICATIONS

### PHOSPHATE INCORPORATION BY STERILE AND NON-STERILE PLANT ROOTS\*

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Bowen and Rovira (1966) have shown in short-term uptake studies that non-sterile clover and tomato plants took up 45 and 85% respectively more phosphate than did sterile plants. Not only was total uptake of phosphate increased in the presence of the rhizoplane microorganisms but translocation of absorbed phosphate to the tops was 4.4 times greater in the non-sterile tomato plants.

These increases in uptake and translocation of phosphate by non-sterile roots were ascribed to the absorption of phosphate by the rhizoplane microflora and to the effects of microorganisms on plant metabolism. It was considered possible that the patterns of phosphate incorporation might also differ, and the results of such studies are reported here.

#### *Materials and Methods*

Seed of wheat (*Triticum vulgare* cv. Gabo) was surface-sterilized with 7% calcium hypochlorite solution, washed with sterile water, and germinated on agar to detect contamination. Sterile germinated seeds (two per tube) were transferred to stainless steel mesh above 40 ml of plant nutrient solution (Hoagland and Arnon 1938) in cotton-plugged test-tubes (3 by 20 cm). Each tube of the non-sterile treatment received an inoculum of 0.1 ml of a 1% soil suspension to provide a wide spectrum of microorganisms. After growth for 7 days in a constant-environment cabinet (12 hr per day at 3000 lumens per square foot, 16°C night and 22°C day), uptake and incorporation experiments were conducted. The sterility of each uninoculated tube was checked immediately before the uptake experiments by transferring an aliquot of 1 ml to a Petri dish containing nutrient agar and incubating at 25°C for 48 hr.

The roots of six individual plants from each of the sterile and non-sterile groups were treated separately to provide replication. Roots of the intact plants were washed in tap water for 60 sec to remove excess plant nutrient solution, placed in  $5 \times 10^{-4}$ M  $\text{CaSO}_4$  solution for 2 hr, and then transferred to a solution of  $5 \times 10^{-4}$ M  $\text{CaSO}_4$  and  $5 \times 10^{-6}$ M  $\text{KH}_2\text{PO}_4$  at pH 6.7 and containing  $[\text{P}^{32}]$ orthophosphate at 400  $\mu\text{C/l}$  at less than  $5 \times 10^{-7}$ M. After 15 min at 20°C the roots were washed in rapidly running water (at 20°C) for 5 min, the tops of the plants removed, and the roots processed as follows. The roots were extracted successively with 0.1N  $\text{HClO}_4$  below 4°C by four successive freezing and thawing cycles during 1 hr to remove the soluble esters and inorganic phosphate, then with 1N  $\text{HClO}_4$  for 18 hr at 4°C, and finally with 0.5N  $\text{HClO}_4$  for

\* Manuscript received May 18, 1966.

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20 min at 70°C. The second and third extracts have previously been designated as "RNA" and "DNA" by Loughman and Russell (1957) but in view of the possibility of cross-contamination they are here considered to represent the total nucleic acid. Extraction volumes were 10 ml followed by three 5-ml washings with the extractant. The residues, containing the phospholipid and the phosphoprotein fractions, were digested with  $\text{HClO}_4$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{HNO}_3$  in the ratio 1 : 1 : 4 (v/v). Aliquots from each fraction were taken for assessing radioactivity in a Geiger-Müller tube adapted for counting liquids. Counts of 2000–20,000 were recorded.

TABLE 1

PHOSPHATE UPTAKE AND PERCENTAGE DISTRIBUTION OF  $^{32}\text{P}$  IN STERILE AND NON-STERILE WHEAT ROOTS

Uptake measured after immersion of roots for 15 min in test solution\*

	Sterile Roots	Non-sterile Roots	S.E. of Mean
Fresh weight (mg)	251	201***	7.3
Phosphate uptake (pmoles/mg fresh wt.)	17.6	23.8***	0.9
Distribution of $^{32}\text{P}$ (%):			
Soluble esters and inorganic phosphate	93.1	89.0**	0.72
Cold 1N $\text{HClO}_4$ extract ("RNA")	1.7	3.3***	0.19
Hot 0.5N $\text{HClO}_4$ extract ("DNA")	1.5	3.8***	0.25
Phosphoprotein and phospholipid	3.7	3.9	0.33

\* See text for experimental details.

\*\* Difference significant at 1% level.

\*\*\* Difference significant at 0.1% level.

## Results

It can be seen from Table 1 that the presence of microorganisms on wheat roots has a marked effect on the patterns of incorporation of phosphate by the roots. Incorporation into the nucleic acid fractions was significantly greater (at 0.1% level) in non-sterile roots than in sterile roots, while the soluble ester-inorganic phosphate fraction was significantly lower in the non-sterile roots. Similar differences have been obtained in the various fractions using 5% trichloroacetic acid for the extraction of acid-soluble esters and inorganic phosphate.

Chromatographic separation by the method of Loughman and Russell (1957) using t-butanol-water-picric acid showed that inorganic phosphate accounted for 57–74% of the acid-soluble fraction, hexose phosphates for 21–28%, and nucleotides for 2–12%. Differences were indicated between sterile and non-sterile roots in their patterns of esterification.

The significant depression of root growth caused by microorganisms (Table 1) is consistent with earlier findings with other plants (Bowen and Rovira 1961). Although non-sterile roots were shorter and lighter than sterile roots, they were more efficient in phosphate uptake (Table 1) with the consequent result that their total uptake was greater.

*Discussion*

Differences between sterile and non-sterile plants in incorporation of phosphate indicate the difficulties of correctly interpreting metabolic patterns in plants unless very stringent precautions are taken to exclude all microorganisms. The strict precautions needed to exclude microorganisms are not realized by many workers. Furthermore in many plant physiological studies it is often thought that washing will reduce the microbial factor to negligible proportions. However, studies with 7-day-old barley roots have shown that counts of bacteria on non-sterile roots before and after washing in rapidly running tap water were  $125 \times 10^6$  and  $2 \times 10^6$ , respectively, per root system. Direct microscopic examination of the 7-day-old wheat roots used in this experiment showed the reduction in numbers due to washing to be similar to that in barley. The data of the experiment reported here were gathered after vigorous washing of the roots. Had the original, denser, rhizoplane population been retained, the difference in the patterns of phosphate incorporation for sterile and non-sterile roots may well have been greater. It is also possible that the differences between sterile and non-sterile roots may be accentuated under conditions for high incorporation into the nucleic acid fractions; Loughman and Russell (1957) found a high incorporation into these fractions when plants of low phosphate status were exposed to low concentrations of phosphate.

The differences shown in this paper between sterile and non-sterile plants may be due simply to the differential incorporation by plant and microbial cells. However, the earlier report (Bowen and Rovira 1966) showed that non-sterile plants translocate a higher proportion of the phosphate adsorbed than do sterile plants, at the phosphate levels used in these investigations. This indicates that there could well be differences in patterns of phosphate incorporation within the roots as well as differences due to incorporation patterns of the microorganisms.

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