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EXPERIMENTAL ARTICLES

Influence of Phosphorus on the Colonization of Barley Rhizosphere by Microorganisms

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Abstract—The mineral phosphorus supply produced two outbreaks in the bacterial population of the barley rhizosphere and rhizoplane but inhibited the growth of fungal mycelium. The inhibition of mycelial growth might be due to the exudation of specific inhibitors by barley roots, since the most pronounced inhibition was observed at high doses of supplementary phosphorus.

Key words: barley, rhizosphere, root, phosphorus, microorganisms

One of the topical problems of soil microbiology is the elucidation of the effects of varying concentrations of deficient nutrients on the colonization of growing roots by different types of microorganisms. Despite the importance of this problem from both the theoretical and practical viewpoints, it is as yet poorly studied.

We have previously shown that one of the specific features of nitrogen as a mineral nutrient of plants [1] is that its application does not essentially accelerate the colonization of plant roots by prokaryotic nitrogen-fixing microorganisms but stimulates their colonization by soil fungi.

Factors affecting the colonization of the rhizosphere and rhizoplane by different microorganisms are poorly understood. At the same time, it is known that some events that take place during plant vegetation, such as significant variations in temperature and soil moisture content or application of fertilizers [2] and pesticides, can change the specificity of different soil microzones, in particular, the rhizosphere and the rhizoplane.

The aim of this work was to study the effect of mineral phosphorus on the colonization of the barley rhizosphere and rhizoplane by the main groups of soil microorganisms.

MATERIALS AND METHODS

Microbial complexes of the barley *Hordeum vulgare* L. var. Tsiklon rhizosphere were studied using a poorly cultivated soddy podzolic soil from the top horizon A. The soil was a weakly differentiated medium loam with a humus content of 1.9%, an absorption capacity of 17.2 mg-equivalent/100 g soil, and pH 6.7 of aqueous extracts. Model experiments were performed in 30-l cultivation vessels each sowed with 80 seeds. The soil moisture content was maintained at a level of 60% of its total moisture capacity, and the soil temperature was 18–20°C. A superphosphate, $Ca(H_2PO_4)_2$, was added in standard and elevated doses of 100 kg/ha (0.03 g/kg soil) and 300 kg/ha (0.1 g/kg soil), respectively, on conversion to P_2O_5.

The total number of microorganisms was determined by luminescence microscopy [3]. For this purpose, the suspensions of soil, rhizosphere, and rhizoplane samples prepared as described by Kirillova [4] were applied by a micropipette onto thoroughly degreased slides in amounts of 0.02 ml per slide for the enumeration of bacteria and 0.04 ml for the enumeration of fungi and uniformly distributed over a slide area of 4 cm^2 by a loop. The material was dried and fixed in the flame of a gas burner. For the analysis of each sample, twelve specimens were prepared. Specimens for the enumeration of bacteria and actinomycetes were stained with an acridine orange solution (1 : 10000) for 2–3 min, and those for the enumeration of fungi, with calcofluor white for 15 min.

Effect of the phosphorus supply on some parameters of barley plants

Parameter	Control	Standard phosphorus dose	High phosphorus dose
Stem length, cm	35.2	38.8	43.8
Spike length, cm	3.0	3.9	4.3
Spike weight, g	0.35	0.39	0.65

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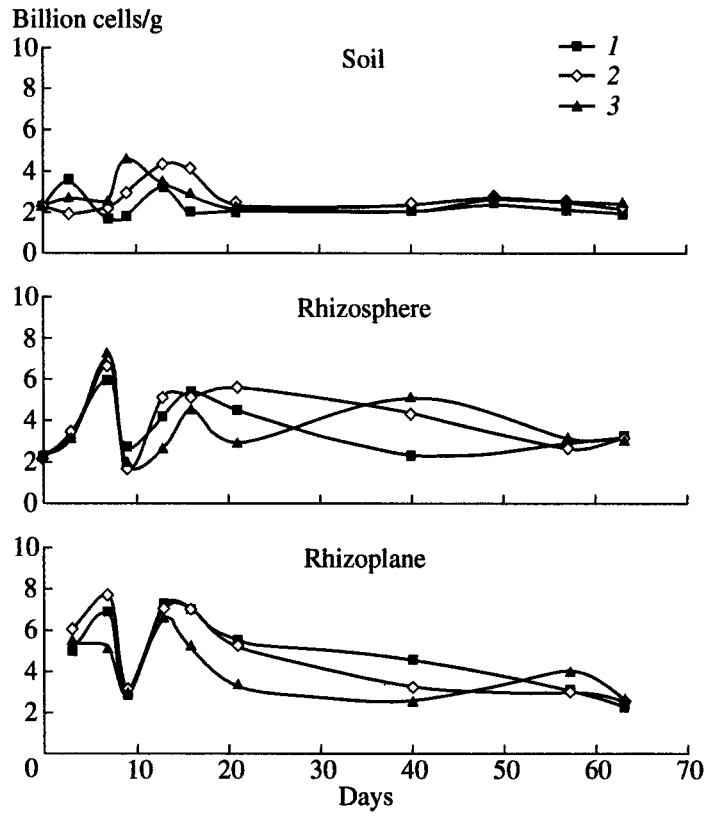

Fig. 1. Dynamics of the bacterial population in the soil, the barley rhizosphere, and the barley rhizoplane (*1*) without and with supplementary phosphorus introduced in amounts of (*2*) 0.03 and (*3*) 0.1 g/kg soil on conversion to P_2O_5.

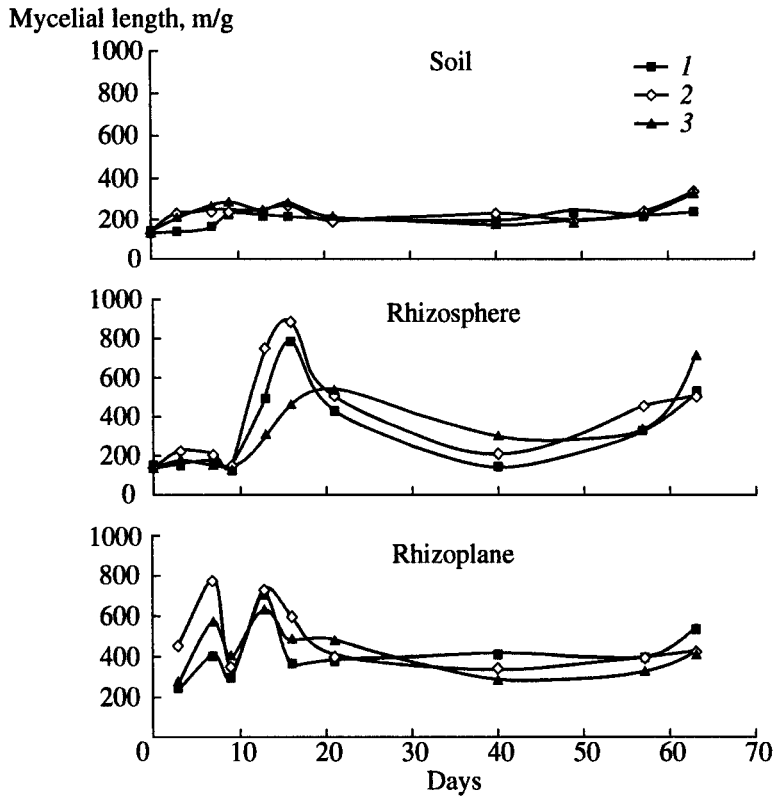

Fig. 2. Dynamics of the length of actinomycete mycelium in the soil, the barley rhizosphere, and the barley rhizoplane with and without supplementary phosphorus. For designations, see Fig. 1.

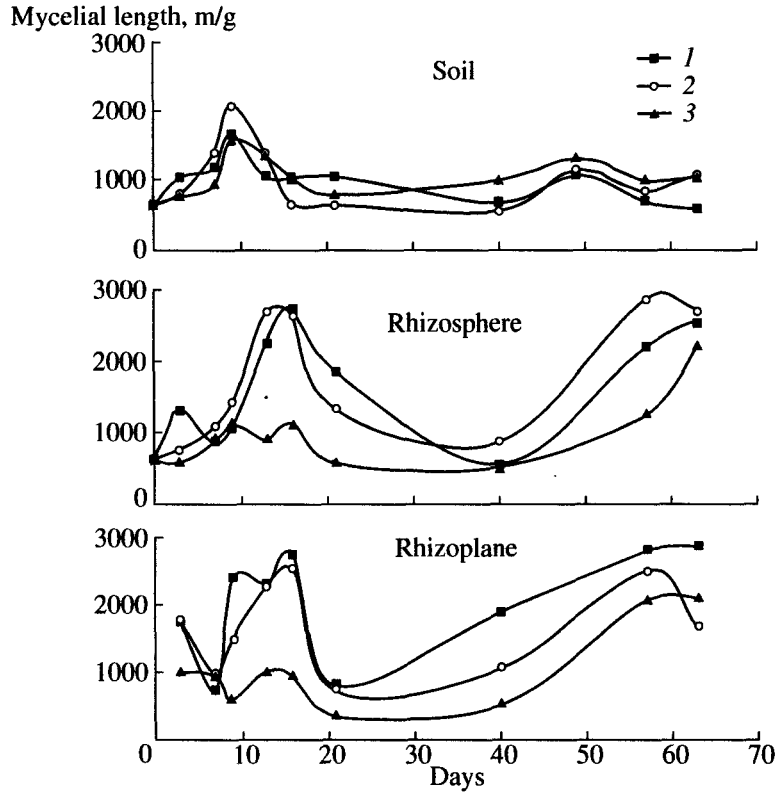

Fig. 3. Dynamics of the length of fungal mycelium in the soil, the barley rhizosphere, and the barley rhizoplane with and without supplementary phosphorus. For designations, see Fig. 1.

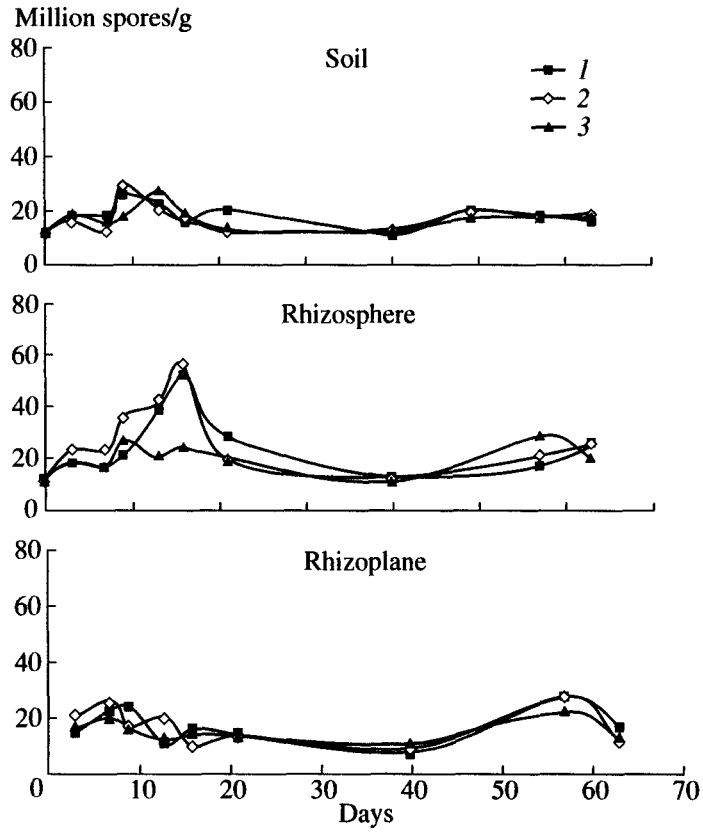

Fig. 4. Dynamics of the number of fungal spores in the soil, the barley rhizosphere, and the barley rhizoplane with and without supplementary phosphorus. For designations, see Fig. 1.

The number of cells (or the amount of mycelia) per 1 g of soil or roots was calculated by the following formula:

$$N = \frac{S_1}{v}\frac{a}{S_2}\frac{n}{c},$$

where N is the number of microbial cells (or mycelium length) per 1 g of soil or roots; S_1 is the area of a specimen expressed in μm^2; a is the number of cells (or mycelium length in μm) in a microscope field averaged over all of the specimens; n is the dilution factor of soil suspensions expressed in ml; v is the volume (in ml) of the droplet applied onto the slide; S_2 is the area of the microscope field in μm^2; and c is the weight (in g) of the soil or root sample.

Standard error did not exceed 5% for the bacterial population and 15% for fungi and actinomycetes.

RESULTS AND DISCUSSION

Phosphorus is considered to be a powerful plant growth–promoting factor [5]. In our experiments, phosphorus caused an increase in the stem length and close to a twofold increase in the crop yield (see table).

The phosphorus content of soil produced no noticeable effect on the shape of the bacterial population curves, although high phosphorus doses increased the bacterial population at the initial stage of succession and then stabilized it at higher levels than in the control (Fig. 1).

In the barley rhizosphere, high phosphorus doses increased the magnitude of the two outbreaks of the bacterial population and coincided with dramatic alterations in the barley root exudation on days 3–7 and 16 of barley growth [6]. Beginning from day 40, the bacterial population stabilized at a level that was maximum in the case of the high dose of introduced phosphate.

High phosphorus doses produced similar outbreaks, albeit much lower, in the bacterial population of the barley rhizoplane. Similar regularities were revealed for the colonization of the barley rhizosphere and rhizoplane by actinomycetes (Fig. 2).

As for fungi (Fig. 3), the stable level of fungal mycelium in the root-free soil was noticeably higher at high than at low phosphorus concentrations, whereas the opposite took place in the rhizosphere and especially in the rhizoplane. In both the rhizosphere and rhizoplane, the standard phosphorus dose favored the growth of fungal mycelium, while high phosphorus doses produced an inhibiting effect. The decrease in the mycelium content of the barley rhizosphere and rhizoplane was accompanied by a decrease in the number of fungal spores in these habitats (Fig. 4).

The results presented may be explained by the reduced role of mycorrhiza in the development of plants under conditions of sufficient phosphorus supply, which may be accompanied by the exudation of corresponding inhibitors by roots [7], although the experimental method used in this work does not allow this suggestion to be proved by the differential count of mycorrhizal and free-living fungi.

The results obtained are in good agreement with the experimental data of Terner *et al.* [2], who revealed the high dependence of the fungal population in the rhizosphere upon the phosphorus-to-nitrogen ratio in the soil. Namely, fungi were most abundant in the rhizosphere at a high supply of nitrogen and a low supply of phosphorus and vice versa.

The data presented should be taken into account in agriculture when using fungicides. In particular, at high doses of introduced phosphorus which are inhibitory to mycorrhiza, fungicides should be applied at lower doses than in the case of insufficient phosphorus supply.

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