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EXPERIMENTAL ARTICLES

Effect of Temperature and Soil Moisture Content on the Colonization of the Wheat Rhizosphere by Antiphytopathogenic Bacilli

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Abstract—Vegetative experiments showed that the population density of antiphytopathogenic bacillar species introduced into the rhizosphere of spring wheat seedlings essentially depended on the soil temperature and not on the soil moisture content. As a rule, the population of introduced bacilli increased with the temperature. Under both low and optimal soil moisture contents, introduced bacilli were efficiently acclimated in the wheat rhizosphere.

Key words: phytopathogen antagonists, rhizosphere, colonization

In countries with developed agriculture, there is increasing interest in biological methods for plant disease control. At present, several biopreparations employing antiphytopathogenic bacilli are known, including Alirin B, Bactophyt, Bacispecin BM, Phytosporin, and Quantum 4000 [1–4]. A significant drawback of these preparations is their unpredictable efficiency under field conditions. Generally, the efficiency of antiphytopathogenic bacterial preparations introduced into the soil is determined by the ability of the component bacteria to acclimate, develop, and maintain their populations in the rhizosphere during the whole period of the vegetative growth of plants. The viability of bacteria introduced into the soil and the rhizosphere depends on environmental conditions, soil moisture content, and temperature in particular. It is believed that the main factor controlling soil microflora is the soil moisture content in the southern and temperate climatic zones and temperature in the northern zones [5]. Little is known about the effect of temperature on antiphytopathogenic bacilli in the plant rhizosphere, although there is evidence that both elevated and low temperatures unfavorably affect bacilli and can diminish their antiphytopathogenic activity [6, 7]. Lengkeek and Otta showed that the optimal growth temperatures of the same bacterial species may considerably differ in vitro and in vivo, and that the mean temperature of wheat seed germination under field conditions does not favor the growth and production of antibiotics by antiphytopathogenic bacteria [8]. For instance, spring wheat seeds begin to germinate at 7–10°C, and many plant pathogens, including the causative agents of root rot, can develop at still lower temperatures, whereas *Bacillus* sp. produce antibiotics only at temperatures higher than 16°C.

The moisture content and the temperature of the soil considerably affect the microbial cenosis of the soil and the rhizosphere, since these factors can essentially change the amount and the pattern of nutrients secreted by plant roots. According to some data, the amounts of root exudates and microorganisms in the rhizosphere rise with the soil moisture content [9]. On the other hand, Reddy and Rahe showed that the population density of *B. subtilis* introduced into the onion seedling rhizosphere depends on the temperature of the soil rather than on its moisture content [7].

The aim of the present work was to estimate the effect of the temperature and soil moisture content on the acclimation of some antiphytopathogenic bacilli in the wheat seedling rhizosphere.

MATERIALS AND METHODS

Genetically marked bacillar strains used in this study—streptomycin-resistant *Bacillus* sp. 739, which is the primary component of the antiphytopathogenic preparation Bacispecin BM, as well as the rifampicin-resistant *B. subtilis* IB-15 and *B. polymyxa* IB-37—were obtained from the collection of the Institute of Biology, Ufa Scientific Center. The efficiency of acclimation and development of strains introduced into the rhizosphere was studied using spring wheat (*Triticum aestivum* L. var. Saratovskaya 55) seedlings.

The influence of the temperature and soil moisture content on introduced bacilli was studied using typical chernozem soil containing (%) humus, 9.28; total nitrogen, 0.61; total P_2O_5, 0.15; CaO, 0.049; and MgO, 0.012 (pH 6.2; C : N = 8.8). To study the effect of temperature, plants were cultivated at 13.0 ± 1.5, 21.0 ± 0.9, and 29.5 ± 0.9°C in soil with the moisture content comprising 60% of the total moisture capacity (TMC).

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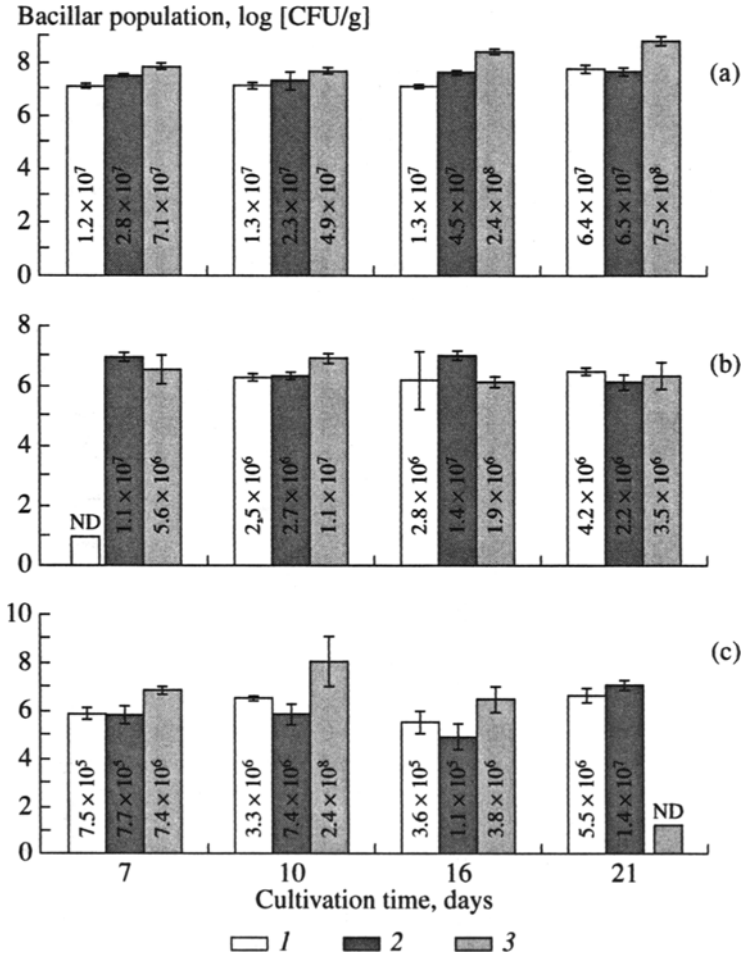

Fig. 1. The population dynamics of introduced bacillar strains in the wheat rhizosphere at different soil temperatures: (a) *B. subtilis* IB-15; (b) *B. polymyxa* IB-37; (c) *Bacillus* sp. 739; (*1*) 13.0°C; (*2*) 21.0°C; and (*3*) 29.5°C. The error bars represent confidence intervals at $P = 0.95$. ND stands for "no data available."

To study the effect of soil moisture, plants were cultivated at 22°C under conditions of insufficient, optimal, and excessive soil moisture contents (37, 67, and 100% TMC, respectively).

Experiments were performed as follows. Bacilli were cultivated in flasks on a rotary shaker (120 rpm) at 37°C for 4 days. The growth medium contained (g/l) starch, 10.0; yeast autolysate, 3.0; corn extract 3.0; peptone, 3.0; $(NH_4)_2HPO_4$, 2.0; K_2HPO_4, 2.0; $(NH_4)_2SO_4$, 2.0; and $CaCO_3$, 5.0 (pH 7.5–7). Spore-containing cells were harvested by centrifugation, washed twice with physiological saline solution, and lyophilized. The bacterial titer of lyophilized preparations was from 10^6 to 10^{10} CFU/g, depending on the strain.

Wheat seeds were sterilized by incubating them in 1% merthiolate for 1 min, washed twice with two volumes of distilled water, and dried on filter paper. Then the seeds were bacterized in proportions of $(6.5–10) \times 10^5$ (*Bacillus* sp. 739), $(1.7–2.8) \times 10^6$ (*B. subtilis* IB-15), and $(0.9–8.9) \times 10^4$ (*B. polymyxa* IB-37) CFU/seed, using Na-carboxymethylcellulose as the glue. Bacterized and sterile (control) seeds were placed onto wet filter paper in petri dishes and allowed to germinate for 3 days. The germinated seeds were transplanted singly into tubes (20 × 200 mm) with soil sterilized as described in the handbook [10] and cultivated in a phytotron with a 16-h illumination period.

The number of bacterial cells in the wheat seedling rhizosphere was determined by plating soil samples onto potato agar with antibiotics and enumerating the number of grown colonies. The results were expressed in CFU per g of fresh roots and rhizospheric soil. The presence of foreign microflora in the soil was checked by plating soil samples onto nutrient and Czapek–Dox agar media. An insignificant contamination of soil with foreign microflora was observed only by the end of the experiments (after 17–20 days of cultivation).

RESULTS AND DISCUSSION

The population dynamics of antiphytopathogenic bacilli in spring wheat rhizosphere at different soil tem-

peratures is shown in Fig. 1. The population of *B. subtilis* IB-15 cells in the rhizosphere virtually did not change during the whole 3-week vegetative period (Fig. 1a), although a small increase in the population of this strain was observed on the 16th day of cultivation at 21 and 29.5°C and on the 21st day of cultivation at 13°C. As a whole, the number of bacterial cells in the rhizosphere increased with the soil temperature and was maximum at 29.5°C. A similar temperature dependence was observed earlier for *B. subtilis* B-2 introduced into the onion seedling rhizosphere: the population of this strain in the rhizosphere was greater at 22–25°C than at 17–19°C [7].

At the same time, the population of *B. polymyxa* IB-37 in the wheat rhizosphere did not show a distinct dependence on the soil temperature: the number of cells of this strain in the rhizosphere varied from 1.9×10^6 to 1.4×10^7 CFU/g without any distinct correlation with the soil temperature or cultivation time (Fig. 1b).

The population of *Bacillus* sp. 739 in the wheat rhizosphere varied from 10^5 to 10^8 CFU/g (Fig. 1c) and showed no noticeable difference at cultivation temperatures of 13 and 21°C. However, at 29.5°C, the number of *Bacillus* sp. 739 cells in the rhizosphere was an order of magnitude higher than at the two other cultivation temperatures.

The maximum number of bacillar cells in the wheat rhizosphere at 13°C was 4.4×10^7 (*B. subtilis* IB-15), 4.2×10^6 (*B. polymyxa* IB-37), and 5.5×10^6 (*Bacillus* sp. 739) CFU/g; therefore, antiphytopathogenic bacilli can acclimate in the wheat rhizosphere at relatively low temperatures. This observation is of great practical importance, since soil phytopathogens infect wheat seedling roots only at low temperatures.

At temperatures of 25–30°C, which are inhibitory to wheat [11], the population density of bacilli in the rhizosphere was also high: 10^7–10^8 (*B. subtilis* IB-15), 10^6–10^7 (*B. polymyxa* IB-37), and 10^6–10^8 CFU/g (*Bacillus* sp. 739), which is also of much importance for the prolonged defence of bacterized plants from phytopathogens. Similar data had been published by Gupta and Utkhede, who observed the maximum production of antifungal compounds by *B. subtilis* AB6 cells in the soil at 21–28°C [12].

The effect of the soil moisture content on the population of introduced bacilli is shown in Fig. 2. At a low moisture content (37% TMC), the number of *B. subtilis* IB-15 cells in the wheat rhizosphere was great (about 10^7 CFU/g) and virtually did not change throughout the experiment (Fig. 2a). At an optimal moisture content (67% TMC), the number of introduced bacillar cells tended to decrease from 1.8×10^7 to 8.9×10^6 CFU/g, whereas at a high moisture content (100% TMC), the number of bacilli increased from 2.9×10^6 to 1.4×10^8 CFU/g.

No significant changes were revealed in the populations of *B. polymyxa* IB-37 in the rhizosphere at low and optimal moisture contents: in both cases, the population density gradually decreased from 10^7 CFU/g on

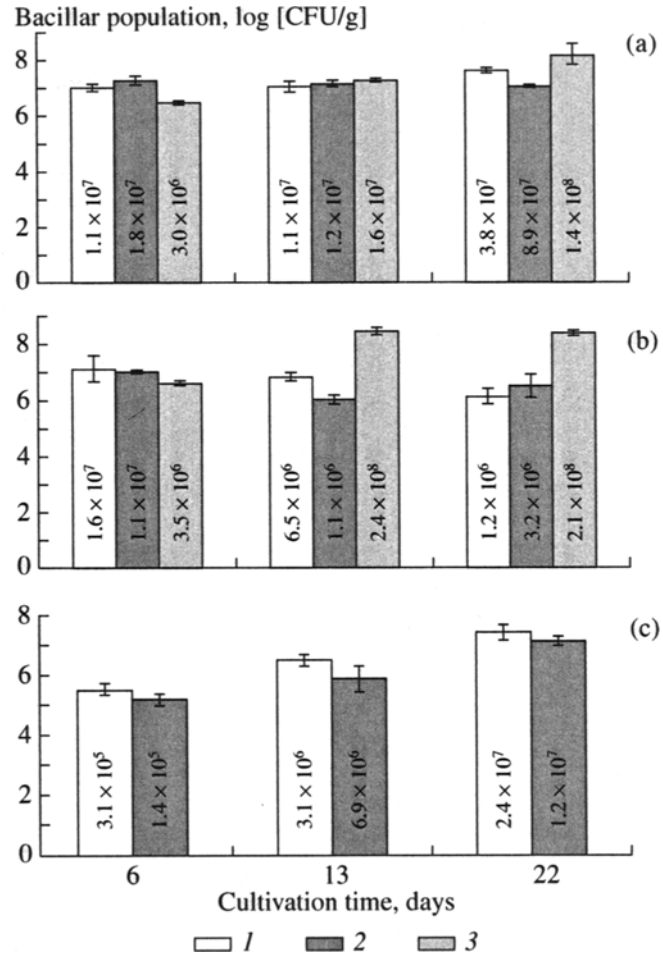

Fig. 2. The population dynamics of introduced bacillar strains in wheat rhizosphere at different soil moisture contents (% TMC): (*1*) 30; (2) 60; and (*3*) 100. For other designations, see Fig. 1.

the sixth day of cultivation to 10^6 CFU/g by the end of the experiment (Fig. 2b). However, at a high moisture content, the population of *B. polymyxa* IB-37 increased from 10^6 to 10^8 CFU/g, showing the same tendency as the population of *B. subtilis* IB-15.

The number of *Bacillus* sp. 739 cells in the wheat rhizosphere was somewhat higher at a low than at an optimal soil moisture content (3.1×10^5–2.4×10^7 and 1.4×10^5–1.2×10^7 CFU/g, respectively) (Fig. 2c). Unlike the populations of *B. polymyxa* IB-37 and *B. subtilis* IB-15, the population of *Bacillus* sp. 739 in the wheat rhizosphere tended to increase at both low and optimal soil moisture contents. These results, together with the data of other authors [13], suggest that the response of introduced bacilli to the soil moisture content is species-dependent. Nevertheless, the soil moisture content is not of crucial importance for the colonization of plant rhizosphere by bacilli, since the metabolism of rhizospheric bacteria is mainly determined by the availability of root exudate, which serves as a source of carbon and energy for these bacte-

Table 1. Effect of soil temperature and seed bacterization on the biomass of wheat plants

Inoculant and soil temperature, °C		Wet plant biomass, mg									
		overground plant parts				roots				Plant biomass by the end of the experiment	Final mass ratio of overground plant parts and roots
		7 days	10 days	16 days	21 days	7 days	10 days	16 days	21 days		
B.subtilis	13	75.6	114.0	204.0	237.4	41.3	52.6	60.3	81.9	319.3	2.9
	21	96.0	188.7	218.0	251.3	63.0	60.7	68.4	84.8	336.1	3.0
	30	111.5	154.8	212.3	245.6	41.3	45.7	56.7	79.3	324.9	3.1
B.polymyxa	13	65.0	91.3	194.0	202.7	39.5	59.5	87.9	119.2	321.9	1.7
	21	73.4	161.3	201.0	240.2	53.5	72.4	98.6	150.1	390.3	1.6
	30	95.0	128.0	151.0	193.4	36.9	53.2	65.8	107.4	300.8	1.8
Bacillus sp.	13	64.0	140.0	174.7	273.5	56.0	57.3	90.3	124.3	397.8	2.2
	21	94.8	187.3	219.3	284.0	63.4	67.0	92.7	129.1	413.1	2.3
	30	93.4	165.7	212.1	263.0	61.3	58.7	83.3	101.1	364.1	2.6
Control	13	54.3	82.6	173.2	201.8	46.3	60.3	86.4	112.1	313.9	1.8
	21	72.0	155.3	181.5	210.4	49.4	68.3	90.6	116.9	327.3	1.8
	30	65.9	132.5	179.5	205.8	43.8	57.9	67.7	102.9	308.7	2.0
$LSD_{0.05}$	13	9.0	13.8	28.9	33.6	5.8	8.5	15.3	14.1	41.4	–
	21	14.4	31.1	36.3	35.1	8.2	9.2	11.6	17.0	62.1	–
	30	14.3	22.1	31.6	35.9	9.2	9.6	12.3	19.3	48.6	–

Table 2. Effect of soil moisture content and seed bacterization on the biomass of wheat plants

Inoculant and soil moisture content, % TMC		Wet plant biomass, mg							
		overground plant part			roots			Plant biomass by the end of the experiment	Final mass ratio of overground plant parts and roots
		6 days	13 days	22 days	6 days	13 days	22 days		
B. subtilis	30	35.0	107.5	244.0	10.4	38.7	90.4	334.4	2.7
	60	66.4	233.0	470.0	18.4	60.7	88.7	558.7	5.3
	100	47.7	135.5	281.0	18.8	54.0	82.6	363.6	3.4
B. polymyxa	30	26.5	121.6	219.0	7.0	28.3	70.6	289.6	3.1
	60	73.2	205.0	451.3	21.4	70.3	132.7	584.0	3.4
	100	38.6	176.5	230.5	9.2	36.0	88.6	319.1	2.6
Bacillus sp.	30	22.0	106.6	255.0	7.5	34.3	115.9	370.9	2.2
	60	57.4	237.6	503.3	19.6	65.7	88.3	591.6	5.7
Control	30	21.4	109.3	205.4	6.8	28.9	114.1	319.5	1.8
	60	79	184.7	326.3	22.0	68.3	90.6	416.9	3.6
	100	35.7	167.2	268.7	10.3	39.4	92.6	361.3	2.9
$LSD_{0.05}$	30	3.6	15.6	37.2	1.4	5.8	10.2	63.9	–
	60	11.3	30.8	46.6	3.1	14.6	12.5	69.5	–
	100	5.9	33.4	26.5	1.7	6.6	9.7	51.6	–

ria. Even when root exudate is the sole source of carbon and energy, the bacterium *B. pumilus* reproduced well on wheat seedling roots in the absence of irrigation [14]. At the same time, there are survival limits for the introduced bacteria under water-deficient and waterlogged conditions. For instance, West *et al.* [15] reported that *B. cereus* and *B. thuringiensis* grew better in wet soil than in dry soil. Furthermore, Campbell and Clor [16] showed that the phytopathogen antagonists *B cereus* var. *mycoides* and *B. pumilus* lost their antiphytopathogenic activity in dry soil and failed to reproduce at soil moisture contents lower than 28% TMC. On the other hand, the proportion of aerobic spore-forming bacilli in irrigated soil decreased in favor of non-spore-forming bacteria [17]. In our experiments, all of the bacillar strains studied were able to survive arid conditions (37% TMC), suggesting that their threshold of drought survival is lower than 37% TMC. It should be noted that the populations of introduced bacilli in the wheat seedling rhizosphere were not statistically different at 37 and 67% TMC.

It is evident that laboratory experiments with the use of axenic soil show only the potentiality of microorganisms to colonize the wheat rhizosphere. In nature, the population density of introduced bacteria will also depend on the activity of indigenous soil biota. In particular, mesofauna can affect introduced microbial populations to a certain degree [18]. The still greater regulatory effect on the introduced bacteria can be exerted by soil protozoa and microflora [19], whose activity depends on the hydrothermal state of the soil.

The population density of rhizospheric microorganisms also correlated with the physiological state of the plants. The data presented in Table 1 show that experimental plants grew well at 21°C but not at 13 or 29.5°C. The mass ratio of the overground parts of the bacterized plants to their roots was dependent on the bacterial species. Namely, this ratio was equal to 3.0, 1.7, and 2.4 for wheat plants inoculated with *B. subtilis* IB-15, *B. polymyxa* IB-37, and *Bacillus* sp. 739, respectively. It is known that the relative mass of plant roots depends on the conditions of mineral nutrition and is regulated by phytohormones. In turn, the phytohormonal status of wheat plants is determined by the presowing bacterization of wheat seeds with antiphytopathogenic bacilli [20]. When grown in liquid cultures, *B. subtilis* IB-15 and *Bacillus* sp. 739, but not *B. polymyxa* IB-37, produce cytokinins [21]. In our opinion, this fact may explain the lower proportion of the overground parts of wheat seedlings bacterized with *B. polymyxa* IB-37 as compared with seedlings treated with the other bacillar strains used. The absence of a correlation between the plant mass and the population density of bacilli introduced into the rhizosphere may imply that these bacilli utilize not only the nutrients of root exudate but also some nutrients from the soil.

The data presented in Table 2 suggest that the physiological state of plants does not directly influence the population density of rhizospheric bacteria. On the other hand, at an optimal soil moisture content, the mass of bacterized plants was 34–42% higher than the mass of the control wheat plants.

Thus, the vegetative experiments performed in this work showed that soil temperature could considerably affect the population density of bacilli in the wheat rhizosphere at the initial stage of development of wheat seedlings. As a whole, the population of introduced bacilli increased with temperature in a species-dependent manner. At the soil temperature at which spring wheat begins to vegetate, the rhizospheric population of all three bacillar strains studied was sufficiently high (from 10^5 to 10^7 CFU/g). Unlike the soil temperature, the soil moisture content was not so essential for the development of introduced antiphytopathogenic bacilli: they actively acclimated in the wheat rhizosphere under both low and optimal soil moisture contents.

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