Microbiology, Vol. 72, No. 4, 2003, pp. 496–502. Translated from Mikrobiologiya, Vol. 72, No. 4, 2003, pp. 554–562.

EXPERIMENTAL ARTICLES

The Abundance and Structure of the Root-Associated Microbial Complexes of Two Greenhouse Rose Cultivars

L. M. Polyanskaya*, S. M. Ozerskaya, G. A. Kochkina**, N. E. Ivanushkina**, A. V. Golovchenko***, and D. G. Zvyagintsev***

**Faculty of Soil Science, Moscow State University, Vorob'evy gory, Moscow, 119899 Russia*

***Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia*

****Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii pr. 33, Moscow, 117071 Russia*

Received January 28, 2002; in final form, June 4, 2002

Abstract—The study of the root-associated microbial complexes of affected and healthy rose plants of two cultivars (Grand gala and Royal velvet) grown in a greenhouse showed that the biomass of eukaryotic microorganisms in the rhizoplane and rhizosphere of healthy rose plants and in the surrounding soil was considerably lower than in the same loci of affected plants. In contrast, the biomass of root-associated prokaryotic microorganisms was higher in the case of healthy than in the case of affected rose plants. The root-associated bacterial complexes of both affected and healthy rose plants were dominated by the genera *Arthrobacter, Rhodococcus*, and *Myxobacterium* and did not contain phytopathogenic bacteria. The root-associated fungal complex of healthy roses was dominated by fungi of the genus *Trichoderma*, whereas that of the affected rose plants was dominated by the species *Aureobasidium microstictum.* The affected cane cuttings and cankers occurring on affected canes were found to contain *Coniothyrium fuckelii* (the causal fungus of rose stem canker) and sclerotia of *Botrytis cinerea* (the causal fungus of gray rot). The micromycete complex of healthy rose plants was not so diverse as was the micromycete complex of affected rose plants.

Key words: soil, plant, microbial complex, structure, rhizosphere, phytopathogens, microbial biomass, biodiversity.

It is well known that root-associated microorganisms can considerably influence the growth of plants and the quality of plant products [1]. It is also recognized that the effect of soil on plants is largely mediated by microorganisms inhabiting the plant rhizosphere [2, 3].

Phytopathogenesis, one of the fundamental problems of microbial ecology [4], is as yet poorly studied. This diminishes the efficiency of the control of phytopathogens and often leads to pesticide overdoses, which calls for intensification of investigations in the field of ecology of microorganisms beneficial or pathogenic to plants [5, 6].

Investigations along this line can be most conveniently conducted in greenhouses, in which the complete account of relevant factors is possible. In this case, the combination of the methods of luminescence microscopy and plating onto selective solid media may yield a great deal of information on the specific interaction of microorganisms with healthy and affected plants [7, 8].

This work was undertaken to comparatively study the root-associated microbial complexes of healthy and affected rose plants with the aim of revealing the specificity of phytopathogenic microbial complexes.

MATERIALS AND METHODS

Samples (a total of 30 plant and soil samples were analyzed) of the rhizoplane and rhizosphere of healthy and affected rose plants of two cultivars (Grand gala and Royal velvet) and soil from depths of 0–5 and 5–10 cm were aseptically collected in a greenhouse of the Ul'yanovskii collective farm in the Moscow region. To identify causal agents, we also sampled the affected bark of Royal velvet rose plants and the cankers girdling affected Grand gala canes.

Rhizosphere and rhizoplane samples were prepared as described by Kirillova [9]. Rose roots with attached soil were placed in 100 ml of sterile tap water and shaken at 180 rpm for 3 min. The suspension of soil thus prepared was considered a sample of the rhizosphere microbial complex. The roots were transferred to the next 100-ml portion of sterile tap water. The microbial cells remaining on the roots were considered a microbial complex of rhizoplane. Soil without rose plants served as the control. All the samples were preliminarily sonicated for 2 min using a UZDN-1 generator (22 kHz, 0.44 A) [10].

The total number of microorganisms was determined by the luminescence microscopy of specimens prepared as described in the handbook [11]. Bacteria

0026-2617/03/7204-0496$25.00 © 2003 MAIK "Nauka / Interperiodica"

and actinomycete mycelium were stained with acridine orange (1 : 10000) for 2–3 min. Yeast cells, fungal spores, and fungal mycelium were stained with calcofluor white for 15 min [12]. Budding yeast cells could easily be differentiated from fungal spores. The standard deviation of count did not exceed 5% for bacteria and 15% for fungal spores, fungal mycelium, and actinomycete mycelium.

Biomass was calculated by assuming that the dry mass of one bacterial cell with a volume of 0.1 μm^3 is 2×10^{-14} g and the dry mass of 1 m of actinomycete mycelium with a diameter of 0.5 μm is 3.9×10^{-8} g. Calculations were carried out by the following formulas: $0.0836r^3 \times 10^{-11}$ g (fungal spores) and $0.628r^2 \times 10^{-6}$ g (fungal mycelium), where r is the radius of fungal spores and hyphae, respectively [13]. The results were expressed in mg/g substrate.

To determine the taxonomic composition of bacteria and actinomycetes, soil suspensions were plated onto selective media [14] supplemented with nystatin (100 mg/l) to inhibit fungal growth. Colonies grown on the plates were counted after 2–4 weeks of their incubation at room temperature. The results were expressed in CFU/g dry substrate. Bacteria were identified based on their morphological, cultural, physiological, and biochemical properties using the manuals [14, 15].

The abundance and the taxonomic composition of fungi were determined by plating cell suspensions on potato–glucose agar (PDA) acidified with lactic acid (4 ml/l) to inhibit bacterial growth. The plates were incubated at 25°C for 5–7 days.

To identify phytopathogenic fungi in the Grand gala rose cane girdle, its fragments were placed onto PDA plates and incubated at room temperature for 7 days and then at 8°C for 30 days. Phytopathogenic fungi in the affected bark of Royal velvet and Grand gala rose canes were identified by essentially the same procedure except that bark fragments were incubated at room temperature for 30 days in a desiccator at a high humidity.

Micromycetes were identified on the basis of their morphological and cultural properties using the identification criteria of Ainsworth and Bisby's Dictionary [16].

RESULTS AND DISCUSSION

Analysis revealed considerable differences between the root-associated microbial complexes of the Grand gala and Royal velvet rose cultivars. The abundance of bacteria and the mass of actinomycete mycelium in the rhizosphere and rhizoplane of the healthy plants of both rose cultivars were 2- to 2.5-fold higher than in the same loci of the affected rose plants (Fig. 1). In the control soil not associated with the rose roots, microorganisms did not show such a distribution.

In contrast, the abundance of fungal spores and cells was higher in the rhizosphere and rhizoplane of the affected rose plants and the surrounding soil than in the same loci of the healthy plants (Fig. 2). The high abundance of fungi is typical of forest floors at high temperatures and moisture contents but not of true soils. Consequently, the high content of fungal spores and yeast cells in a soil may serve as an indication of phytopathogenic processes in the plants growing in this soil, since some microloci in the plant rhizosphere may be favorable for the germination of spores of phytopathogenic fungi.

The content of fungal mycelium in the rose rhizosphere, rhizoplane, and the surrounding soil was relatively low, especially in the case of healthy plants.

The girdles exhibited a high abundance of bacteria, likely phytopathogenic (Table 1), the biomass of bacteria being about 8 times that of fungi. Inasmuch as bacteria can be involved in the pathogenesis of plant tissues, they may enhance the impact of phytopathogenic fungi. Analysis showed that the affected rose cuttings contained little bacteria (1%) but much fungal mycelium (48%) and many fungal spores and yeast cells (51%).

The microbial biomass, including the mass of fungal mycelium, fungal spores and yeast cells, in the rhizosphere, rhizoplane, and the surrounding soil of the healthy plants of both rose cultivars was 1.5 to 2 times lower than in the same loci of the affected plants (Fig. 3). As for the differences between the cultivars, the microbial biomass was at a maximum in the rhizosphere of the Royal velvet roses and at a minimum in the surface layer of the soil surrounding these roses. In the deeper soil layer, the cultivar-dependent differences between the microbial complexes were negligible.

The root-associated prokaryotes (bacteria and actinomycete mycelium) were more abundant in the rhizosphere and the rhizoplane of the healthy Grand gala plants (38%) than in the same loci of the healthy Royal velvet plants (19%).

Comparative study showed that the rhizoplane of the healthy rose plants contained 2 to 6 times more saprotrophic bacteria than did the rhizoplane of the affected rose plants and that these bacteria were more abundant in the Grand gala rhizoplane (1200 to 7800 million CFU/g) than in the Royal velvet rhizoplane (200–400 million CFU/g) (Table 2).

The population density of saprotrophic bacteria decreased in the direction from the rhizoplane, being minimum in the subsurface layer (5–10 cm) of the surrounding soil. In this locus, bacterial abundance was almost independent of the condition of plants and varied, depending on the distance from the plant roots, from 270 to 420 million CFU/g soil (Grand gala) and from 40 to 360 million CFU/g soil (Royal velvet) (Table 2).

Analysis showed that bacteria and actinomycetes were more abundant in loci with a high content of phytopathogens (i.e., in the girdles). Indeed, the average density of bacteria on the surface of the affected rose canes was 20 million CFU/g, while reached 150 million CFU/g in the girdles.

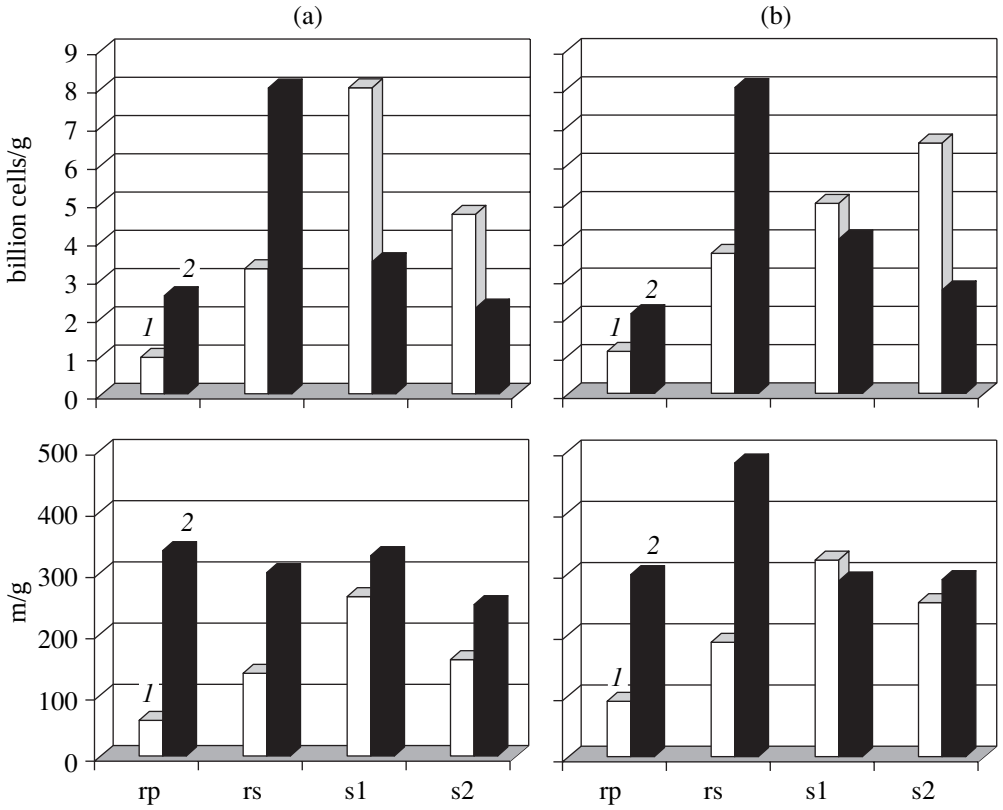

Fig. 1. The abundance of bacteria (billion cells/g) and the total length of actinomycete mycelium (m/g) in the rhizoplane (rp), rhizosphere (rs), and soil at depths of 0–5 cm (s1) and 5–10 cm (s2) of (1) the affected and (2) healthy plants of the rose cultivars (a) Grand gala and (b) Royal velvet.

The investigation of the taxonomic composition of bacteria in the girdles from the affected Grand gala rose canes showed the girdles contained harmless bacteria of the genus *Aquaspirillum* (Fig. 4), which are ubiquitous inhabitants of aquatic ecosystems and hydromorphic soils. These bacteria could come to the greenhouse soil and plants with irrigation water and multiply in the soil, rhizosphere, and especially girdles, where aquaspirilla can utilize sugars and organic acids liberated from plant tissues by phytopathogenic bacteria. The other bacteria isolated from the girdles were minor and also nonphytopathogenic (Table 4).

The surface of the affected rose canes beyond lesions was dominated by nonphytopathogenic coryneform bacteria of the genus *Brevibacterium*, which do not posses hydrolytic activity but can multiply as epiphytes in the phylloplane due to their ability to utilize amino acids and sugars. These bacteria are common inhabitants of dairy products and wastewaters and could come to the greenhouse together with soil.

Table 1. The abundance and biomass of microorganisms in the bark and girdle of the affected rose plants

Sample	Microorganisms	Abundance	Biomass, mg/g	Percent
Girdle on affected Grand gala cane	Bacteria	8.0 billion cells/g	0.4	89.0
	Actinomycete mycelium	0	0	0
	Fungal mycelium	38.0 m/g	0.03	7.0
	Fungal spores and yeast cells	2.4 million cells/g	0.02	4.0
Bark of affected Royal velvet cane	Bacteria	0.4 billion cells/g	0.01	1.0
	Actinomycete mycelium	0	0	0
	Fungal mycelium	180.0 m/g	0.30	48.0
	Fungal spores and yeast cells	20.0 million cells/g	0.32	51.0

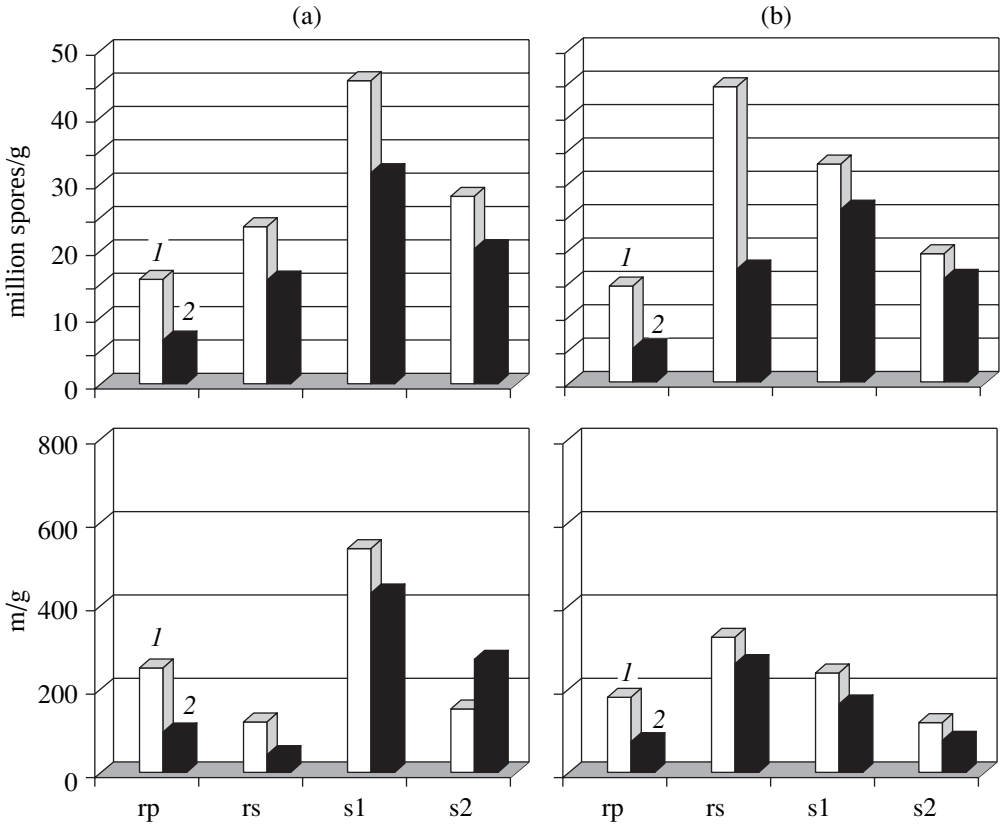

Fig. 2. The content of fungal spores and yeast cells (million/g) and the total length of fungal mycelium (m/g) in the rhizoplane (rp), rhizosphere (rs), and soil at depths of 0–5 cm (s1) and 5–10 cm (s2) of (*1*) the affected and (*2*) healthy plants of the rose cultivars (a) Grand gala and (b) Royal velvet.

The taxonomic structure of the bacterial complexes from the rose roots and the surrounding soil is shown in Tables 3, 4. The rhizoplane of the healthy and affected Grand gala rose plants was dominated by myxobacteria, spirilla, and corineform bacteria and contained, as minor components, such soil bacteria as azotobacter, cytophages, bacilli, and streptomycetes. The bacterial complex of the Royal velvet rhizoplane did not contain dominants and was more diverse than the rhizoplane bacterial complex of Grand gala, indicating that soil under this cultivar was more favorable for bacterial growth. The rhizoplane bacterial complexes of the healthy and affected plants of both rose cultivars did not show any notable taxonomic differences.

The content of corineform bacteria, dominated by the genera *Arthrobacter* and *Rhodococcus*, increased in the surrounding soil (as compared with the rhizoplane). Some soil samples contained many azotobacters. The soil under the Royal velvet rose cultivar was characterized by a high content of myxobacteria.

The general vertical trend in the fungal abundance of soil was a downward decrease from (0.53–2.9) × 10^6 CFU/g soil in the 0–5 cm layer to (0.5–3.0) × 10^5 CFU/g soil in the 5–10 cm layer (Table 3).

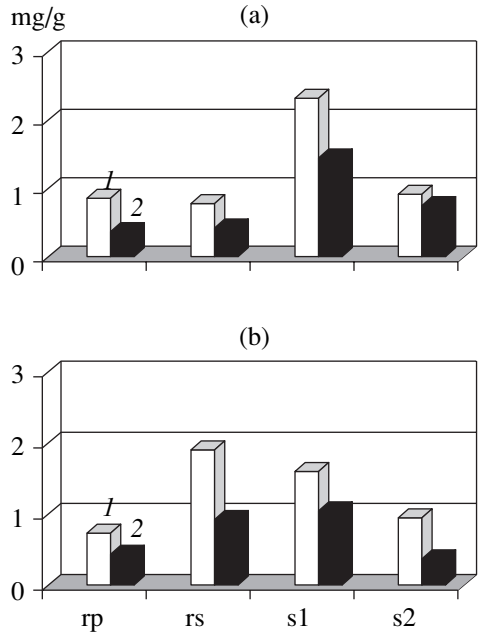

Fig. 3. Microbial biomass (mg/g) in the rhizoplane (rp), rhizosphere (rs), and soil at depths of 0–5 cm (s1) and 5–10 cm (s2) of (*1*) the affected and (*2*) healthy plants of the rose cultivars (a) Grand gala and (b) Royal velvet.

Table 2. The average number of bacteria in the rose rhizoplane and the surrounding soil (million CFU/g)

Cultivar	Sample	Rhizoplane	Soil, 0–5 cm	Soil, 5–10 cm
Grand gala	Affected plants	1262.5	401.5	312.0
	Healthy plants	7849.0	418.0	269.0
Royal velvet	Affected plants	230.5	287.6	40.0
	Healthy plants	458.0	356.0	62.0

The girdles of the affected Grand gala plants contained less fungal propagules than did the bark and the rhizoplane of the same plants. The soil under healthy plants contained less fungi than did the soil under affected plants ((2.1–8.2) × 10^4 CFU/g and (5–30) × 10^4 CFU/g, respectively).

The species composition of microscopic fungi differed in different rose cultivars, which was likely due to the different types of soil used for their cultivation. For instance, the presence of zygomycetes (*Mortierella*, *Mucor*, and *Rhizopus*) in the soil under the Grand gala cultivar could be due to the fact that this soil was fertilized with manure. The fungal complex of the Grand gala cultivar was more diverse (12 genera) than the fungal complex of the Royal velvet cultivar (10 genera). Fungi of the genera *Aspergillus*, *Geotrichum*, and *Aureobasidium* were found only in the fungal complex of Grand gala. The genus *Aureobasidium* was represented by the only species *A. microstictum* (Bubak) W.B. Cooke, which is slightly phytopathogenic and, hence, its predominance in the rhizoplane and girdles (35–60%) and in the surrounding soil (more than 70%) may indicate that the growth conditions in the greenhouse are unfavorable to the Grand gala cultivar.

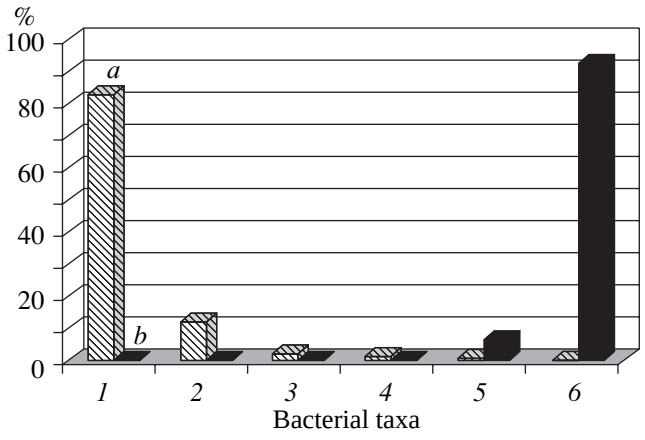

Fig. 4. The taxonomic composition of bacterial complexes on the affected canes and in the girdles of the rose cultivars (*a*) Grand gala and (*b*) Royal velvet: *1*, spirilla; *2*, myxobacteria; *3*, rhodococci; *4*, bacilli; *5*, yellow corineform bacteria; *6*, other corineform bacteria.

All the samples under study contained some known species of the genus *Penicillium*. The genus *Acremonium* dominated in the bark of the affected Royal velvet plants (about 90%). The soil under this cultivar contained fungi of the genus *Fusarium* (13.7–16.7%) and *Gilmaniella* (16.7%). The roots of dead plants showed the presence of fungi of the genus *Paecilomyces* (7.2%). Fungi of the genus *Trichoderma* were detected in the soil under the Royal velvet cultivar (50–100% of the total fungi) and in all plant samples except for the affected bark.

Analysis of the complex of mycelial fungi activated by incubating the affected plant tissues as described in the *Material* and *Methods* section showed that the affected rose plants were contaminated by the phytopathogenic fungi *A. microstictum, Botrytis cinerea* Persoon : Fries, and *Coniothyrium fuckelii* Saccardo and by the microscopic fungi *Doratomyces stemonitis* (Persoon : Fries) Morton et G. Smith, *Aspergillus* spp., *Acremonium* spp., and *Cladosporium* spp.

Thus, our investigations showed that prokaryotic microorganisms were more abundant in the rhizosphere and rhizoplane of the healthy rose plants than in the same loci of the affected plants. This may favorably influence the physiological state of rose plants, since prokaryotes produce many metabolites and some can fix nitrogen into forms available to plants. The high content of fungi, some of which may appear to be phytopathogenic, in the surrounding soil is not beneficial to plants.

The micromycete complexes of the affected rose plants were more diverse than the complexes of the healthy plants (Table 5). The surface layer (0–5 cm) of the soil under the affected Grand gala rose plants was inhabited by seven genera of mycelial fungi, including the phytopathogenic genera *Aureobasidium* (the species *A. microstictum*) and *Geotrichum*, whereas the same surface soil layer under the healthy plants was inhabited by fungi of four genera. The presence of the phytopathogenic species *A. microstictum* and micromycetes of the genera *Penicillium*, *Mucor*, and *Rhizopus* in the soil under the affected rose plants may diminish their immunity. In contrast, the soil under the healthy rose plants was dominated by the *Trichoderma* fungi.

Much the same distribution patterns were observed for the Royal velvet rose cultivar. The subsurface layer (5–10 cm) of the soil under the affected Royal velvet plants was inhabited by mycelial fungi of five genera. At the same time, the population of soil micromycete under the healthy Royal velvet plants was almost totally dominated by the *Trichoderma* fungi, which are known as antagonists of phytopathogenic fungi and serve as the principal of the antifungal preparation Trichodermin [17].

Table 3. The taxonomic composition of bacterial complexes in the rhizoplane and soil of (A) the healthy and (B) affected Grand gala rose plants

Plant	Sample	Relative abundance of taxa, %						
		1	2	3	4	5	6	7
A	Rhizoplane	2.0	75.8	0	0	0	14.4	3.7
	Soil, 0–5 cm	0	14.2	5.4	10.4	1.7	5.8	62.5
	Soil, 5–10 cm	0	25.9	0	0	2.3	2.3	69.5
B	Rhizoplane	0	35.9	7	28	3.6	10.5	15
	Soil, 0–5 cm	43.1	14.7	1.8	0	4.4	10.8	25.2
	Soil, 5–10 cm	27.1	3.2	0	0	13.4	18.2	38.1

Note: 1, azotobacter; 2, myxobacteria; 3, cytophages; 4, spirilla; 5, bacilli; 6, streptomycetes; 7, corineform bacteria.

Table 4. The taxonomic composition of bacterial complexes in the rhizoplane and soil of (A) the healthy and (B) affected Royal velvet rose plants

Plant	Sample	Relative abundance of taxa, %						
		1	2	3	4	5	6	7
A	Rhizoplane	0	10.4	4.2	10.2	28.9	18.8	27.5
	Soil, 0–5 cm	0	37.9	0	0	13.5	2.5	46.1
	Soil, 5–10 cm	0	17.7	0	0	28.6	17.9	35.8
B	Rhizoplane	0	19.2	0	7.9	22.6	35.2	15.1
	Soil, 0–5 cm	0	47	0	0	15.6	15.6	21.8
	Soil, 5–10 cm	39	20	0	0	10	12.5	18.5

Note: 1, azotobacter; 2, myxobacteria; 3, cytophages; 4, spirilla; 5, bacilli; 6, streptomycetes; 7, corineform bacteria.

Table 5. The abundance and generic composition of microscopic fungi in the rose rhizoplane and the surrounding soil as estimated by the plate method

Rose cultivar	Plant	Sample	CFU/g	Relative abundance of fungi, %														
				Mortierella	*Mucor*	*Rhizopus*	*Penicillium*	*Geotrichum*	*Trichoderma*	*Aspergillus*	*Fusarium*	*Paecilomyces*	*Acremonium*	Mycelia sterilia (dark)	*Aureobasidium*	*Phoma*	*Cladosporium*	*Gilmaniella*	Unidentified fungi
Grand gala	Affected	Girdle	3.5×10^5				48.1			1.3					35.4	12.7			2.5
		Roots	2.1×10^6	0.9	1.6		65.7							3.2	15.6	13.0			
		Soil, 0–5 cm	2.9×10^6		4.4	1.5	19.5	1.4						4.4	50.4		8.5		9.9
		Soil, 5–10 cm	3.0×10^5				40.5		3.4						5.6				50.5
	Healthy	Roots	7.1×10^5				42.9							14.3	14.3		28.6		
		Soil, 0–5 cm	5.2×10^5				76.4		5.9					5.9			11.8		
		Soil, 5–10 cm	8.2×10^4				33.4		16.7					33.3					16.7
Royal velvet	Affected	Bark	3.5×10^6				12.8						87.2						
		Roots	1.1×10^6				20.7		18.2			7.2		1.3			2.6		50.2
		Soil, 0–5 cm	5.3×10^5				35.3		5.0					5.5			12.6		37.5
		Soil, 5–10 cm	5.0×10^4						4.6		13.7			37.9		13.6	12.6		13.6
	Healthy	Roots	2.3×10^5				11.8		5.9					41.2			35.3		5.9
		Soil, 0–5 cm	6.9×10^4						50.0		16.7							16.7	16.7
		Soil, 5–10 cm	2.1×10^4						100.0										

The high diversity of micromycetes (both phytopathogenic and saprotrophic) in the greenhouse soil may bring about phytopathogenic processes. Consequently, the abundance and diversity of fungal propagules in soil can serve to monitor its "health."

The analysis of micromycetes in the girdles of the affected Grand gala rose plant showed that they contained sclerotia of *Botrytis cinerea* (the causal fungus of gray rot) and some *Penicillium* and *Aspergillus* fungi, which are not phytopathogenic and were unlikely to be related to the plant disease.

The cuttings of the affected rose plants were inhabited by two phytopathogens, *B. cinerea* and *C. fuckelii* (the causal fungus of rose stem canker), which suggested a combined infection of these plants. The fungus *D. stemonitis* identified on the bark of the affected plants was evidently a secondary inhabitant of the dying-out plant tissues.

Thus, the monitoring of the state of the root-associated microbial complex of plants may be used for making decision as to the application of costly agrochemicals. In addition to the known agronomic methods, such as plant treatment with fungicides and the removal of severely affected plants and dead root systems, some other approaches can be proposed. Among them, the application of chitin as a substrate for the development of antagonistic actinomycetes [13], the enrichment of soil with phosphorus, which stimulates the development of beneficial soil bacteria [18], and the live preparation Trichodermin, whose principal *Trichoderma* fungi controls the development of phytopathogenic fungi [17].

ACKNOWLEDGMENTS

This work was supported by grant nos. 00-04-48029 and 03-04-48620 from the Russian Foundation for Basic Research.

REFERENCES

1. Whipps, J.M. and Lynch, J.M., The Influence of the Rhizosphere on Crop Productivity, *Adv. Microb. Ecol.*, 1986, vol. 9, pp. 187–244.
2. Rovira, A.D., Foster, R.S., and Martin, J.K., Note on Terminology: Origin, Nature, and Nomenclature of the Organic Materials in the Rhizosphere, *The Soil–Root Interface*, Harley, J.L. and Russel, R.S., Eds., London: Academic, 1979, pp. 145–160.
3. Foster, R.C., The Biology of Rhizosphere, *Ecology and Management of Soil-borne Plant Pathogens*, St. Paul Minn., 1985, pp. 75–79.
4. Click, B.R. and Bashan, Y., Genetic Manipulation of Plant Growth-promoting Bacteria to Enhance Biocontrol of Phytopathogens, *Biotechnol. Adv.*, 1994, vol. 15, no. 2, pp. 353–378.
5. Liljeroth, E., Baath, E., Mathiasson, I., and Lundborg, T., Root Exudation and Rhizoplane Bacterial Abundance of Barley (*Hordeum vulgare* L.) in Relation to Nitrogen Fertilization and Root Growth, *Plant Soil*, 1990, vol. 127, pp. 81–89.
6. Youssef, R.A., Kanazawa, S., and Chino, M., Distribution of Microbial Biomass across the Rhizosphere of Barley (*Hordeum vulgare* L.) in Soils, *Biol. Fertil. Soils*, 1989, vol. 7, pp. 341–345.
7. Polyanskaya, L.M., Orazova, M.Kh., Sveshnikova, A.A., and Zvyagintsev, D.G., The Effect of Nitrogen on the Microbial Colonization of the Barley Rhizosphere, *Mikrobiologiya*, 1994, vol. 63, no. 2, pp. 308–313.
8. Ozerskaya, S.M., The Composition of Soil Micromycetes in Two Mixed Forest Biogeocenoses, *Cand. Sc. (Biol.) Dissertation*, Moscow: Moscow State University, 1980.
9. Kirillova, N.P., Microbial Population Dynamics in Model Soil–Plant Systems, *Cand. Sc. (Biol.) Dissertation*, Moscow: Moscow State University, 1983.
10. Zvyagintsev, D.G., Dmitriev, E.A., and Kozhevin, P.A., On the Luminescence Microscopy of Soil Microorganisms, *Mikrobiologiya*, 1978, vol. 47, pp. 1091–1096.
11. *Metody pochvennoi mikrobiologii i biokhimii* (Methods of Soil Microbiology and Biochemistry), Moscow: Mosk. Gos. Univ., 1991.
12. Polyanskaya, L.M., Direct Microscopic Count of Fungal Spores and Mycelium in Soil, *Tez. konf. "Izuchenie gribov v biogeotsenozakh"* (Proc. of Conf. "The Study of Fungi in Biogeocenoses"), Sverdlovsk, 1988, p. 30.
13. Polyanskaya, L.M., Microbial Succession in Soil, *Doctoral Dissertation*, Moscow State University, 1996.
14. Dobrovol'skaya, T.G., Skvortsova, I.N., and Lysak, L.V., *Metody vydeleniya i identifikatsii pochvennykh bakterii* (Methods for the Isolation and Identification of Soil Bacteria), Moscow: Mosk. Gos. Univ., 1989.
15. *Bergey's Manual of Systematic Bacteriology*, Krieg, N.R. and Holt, J.G., Eds., London: Williams & Wilkins, 1984.
16. *Ainsworth and Bisby's Dictionary of the Fungi, Hawksworth et al.*, Eds., 8th ed., Cambridge: Univ. Press, 1995.
17. Misko, L.A., *Rozy. Bolezni i zashchitnye meropriyatiya* (Roses: Diseases and Their Control), Moscow: Nauka, 1986.
18. Orazova, M.Kh., Burkanova, O.A., Polyanskaya, L.M., and Zvyagintsev, D.G., Influence of Phosphorus on the Colonization of Barley Rhizosphere by Microorganisms, *Mikrobiologiya*, 2000, vol. 69, no. 3, pp. 420–425.