V. A. Lyakh*, N. I. Kostyuchenko and I. A. Shevchenko Broomrape (*Orobanche cumana* Wallr.) can Influence the Microbial Cenosis in Sunflower Rhizosphere

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Abstract: The bacterial and micromycete complexes in the rhizosphere of sunflower plants non-infected and infected with broomrape (Orobanche cumana Wallr.) have been compared. The investigations were carried out in the conditions of a stationary infectious field which was annually enriched with infected plant residues and broomrape seeds collected in different regions of Ukraine. Soil is leached, low-humic chernozem with acidic pH. The soil samples selected at the end of vegetation from the rhizosphere of healthy and infected with broomrape plants of sunflower breeding samples. The total number of bacteria found in the rhizosphere of sunflower plants infected by the parasite did not differ significantly from the control and was 11.7 and 12.1 million CFU / g of soil, respectively. The numbers of ammonifiers as well as bacterial microflora, using for its life mineral nitrogen, and pedotrophs and oligotrophs in the compared soil samples did not differ significantly, and generally corresponded to this type of soil. Although in general, both tested samples of soil were characterized by a low content of bacteria of the genus Azotobacter, the number of representatives of this genus in the rhizosphere of parasite-infected plants was somewhat less than in control (35% and 21%, respectively). However, unlike most bacteria, the number of micromycetes detected on Czapek-Dox and starch-ammonia agar media, in the rhizosphere of plants infected by broomrape almost twice exceeded the number of these microorganisms in the rhizosphere of healthy plants. Analysis of the generic and species composition of microscopic fungi showed that in the rhizosphere of sunflower plants infected by the parasite a very specific mycocenosis was formed that differ from a mycocenosis of healthy plants. This mycocenosis was characterized by a much smaller number of genera and species of micromycetes. At the same time for the structure of the fungal cenosis of

^{*}Corresponding author: V. A. Lyakh, Biological Faculty, Zaporozhye National University,

Zhukovskogo str., 66, Zaporozhye 69600, Ukraine, E-mail: lyakh@iname.com

N. I. Kostyuchenko, Biological Faculty, Zaporozhye National University, Zhukovskogo str., 66, Zaporozhye 69600, Ukraine, E-mail: kostuchenko.zp@gmail.com

I. A. Shevchenko, Institute of Oilseed Crops of NAAS, Institutskaya Str., 1, Zaporizhzhia 70417, Ukraine, E-mail: shevchenko_i@meta.ua

diseased plants there was a characteristic increase in the proportion of toxinforming fungi of the *Aspergillus* and *Penicillium* genera with active conidiogenesis. The obtained data testify not only to the differences in the microbial complexes in the rhizosphere of sunflower plants non-infected and infected by broomrape, but also indicate the direction of action of this parasite.

Keywords: sunflower, broomrape, rhizosphere, microbial complex, mycocenosis

Introduction

Intensification of sunflower cultivation in many countries as a highly profitable oilseed crop has led to the emergence and rapid spread of broomrape (*Orobanche cumana* Wallr.), which is considered one of the most dangerous factors affecting the production of this crop (Kaya, 2014).

Broomrape is an obligate parasite from the higher flowering plants of the *Orobanchaceae* family. It does not have its own roots and is completely devoid of chlorophyll. Plants of broomrape parasitize on the roots of sunflower plants, which leads to a significant decrease in yield, deterioration in the quality of seeds, and sometimes even the destruction of crops (Pacureanu-Joita *et al.*, 2012).

Seeds of broomrape grow under the influence of root secretions of sunflower. Settled on the roots of plants, broomrape dehydrates and depletes sunflower. Therefore, the parasite causes maximum damage under arid conditions, exacerbating the effect of drought (Antonova *et al.*, 2012). Potentially immunity to broomrape in sunflower can be expressed in the absence of its roots in the ability to stimulate the germination of parasite seeds. During the conjugate evolution with sunflower, new virulent races periodically arise in the parasitic plant, overcoming the immunity of the existing genotypes of this crop. Currently, eight races of *O. cumana* (A, B, C, D, E, F, G, H) are parasitic on sunflower. The most virulent G and H races, which affect sunflower hybrids resistant to all preceding races, have already been found in Spain, Romania, Turkey and a number of other European countries (Fernandez-Martinez *et al.*, 2008; Kaya *et al.*, 2004; Melero-Vara *et al.*, 2000; Pacureanu-Joita *et al.*, 2012; Shindrova, 2006).

It is known that root exudates of plants selectively affect the composition of the microbial cenosis, acting as a growth substrate for soil microorganisms. It has even been shown that, according to the changes in the composition of exudates in different zones of the root of the plant, the species composition and structure of the microbial communities of the rhizosphere of different sites can vary. In turn, the living conditions of plants and the productivity of phytocenoses depend on the structure and physiological activity of the soil microorganism complex (Badri and Vivanco, 2009; Bais *et al.*, 2006; Mahaffee and Klopper, 1997; Yang and Crowley, 2000).

The composition of root exudates and, as a result, the species composition and structure of the microbial community largely depend on the nutritional conditions of plants affected by soil type, plant age, diseases, various stress factors (Kurdish, 2010). One of such stress factors is broomrape, which, growing into the roots of sunflower plants, takes away water and nutrients from them, thus often depressing them to complete destruction.

At present, much is known about the features of the structure of microbial complexes in the rhizosphere of many agricultural plants (Babayants, 2011; Belyuchenko, 2016; Haldar and Sengupta, 2015), including sunflower (Kostyuchenko and Lyakh, 2017). However, there is practically no information on whether such a root flowering parasite as a broomrape can affect a complex of microbes that is typical of the rhizosphere of sunflower, settling on its roots.

The aim of this study was to compare the structure of the bacterial and micromycete complex in the rhizosphere of infected with broomrape (*O. cumana*) and non-infected sunflower plants, which would give an opportunity to assess the direction of the parasite effect.

Materials and methods

The investigations were carried out in the conditions of a stationary infectious nursery of the Institute of Oilseed Crops (IOC) of NAAS. This nursery was set in 2005 to assess the breeding samples of oilseeds to a complex of diseases. Each year, it was enriched with infectious material (infected plant residues and broomrape seeds) collected in different regions of Ukraine.

Agrotechnics of growing sunflower is common for the conditions of the south of Ukraine. In the experiment, sunflower seeds were sown in a square-nested manner (70 cm \times 70 cm), leaving one plant in the nest. Soil is leached, low-humic chernozem (humus content is 3.0–4.0%), pH – 5.6. Total nitrogen content was 0.2%, absorbed ammonium nitrogen – 18 mg / kg and total phosphorus – 16 mg / kg.

The soil samples selected at the end of September 2017 just before harvesting from the rhizosphere of healthy and infected with *O. cumana* plants of sunflower breeding samples served as the material for the study. Broomrape plants at that time were at the stage of ripened capsules.

Sampling of soil, as well as isolation, cultivation, registration of microbial complexes was carried out according to generally accepted methods (Mirchink, 1988; Zvyagintsev, 1991).

Samples of rhizospheric soil (combined samples of 10 plants) from infected and non-infected (control) plants were selected three times. The repetition of microbiological experiments is fivefold.

To isolate bacteria and microscopic fungi from the soil, a conventional method of serial dilutions was used, followed by seeding the soil suspension onto nutrient media (Dudka *et al.*, 1982).

Optimal nutrient media were used to account for the number of basic ecological-trophic groups of microorganisms: for ammonifiers – meat-peptone agar (MPA); for bacteria utilizing mineral nitrogen – starch-ammonia agar (SAA); for pedotrophs – soil agar (SA); for oligotrophs – 'hungry' agar (HA); for bacterium of the *Azotobacter* genus – Ashby medium; for the microscopic fungi used Czapek-Dox medium with sucrose and starch-ammonia agar (SAA). The duration of cultivation is 5–14 days at a temperature of 28 °C.

The number of microorganisms was expressed in colony-forming units (CFU) in 1g of air-dry soil. The number of bacteria of the genus *Azotobacter* was determined by the percentage of soil lumps fouling on Ashby medium.

To evaluate the activity of microbiological processes taking place in the investigated soil, the following coefficients were used: mineralization-immobilization, calculated by the ratio of the number of microorganisms using mineral and organic nitrogen (SAA / MPA); oligotrophy – by the ratio of the number of oligotrophs growing on poor media to the total number of bacteria in SAA and MPA; pedotrophy – by the ratio of microorganisms on soil agar to the number of microorganisms grown on MPA (SA / MPA).

To determine the similarity of the species composition of the microbiota, the Sorensen similarity coefficient (Cs) was calculated (Megarran, 1992).

Identification of fungi carried out using handbooks and original works (Bilaj and Koval, 1988; Domsh *et al.*, 1993; Hoog *et al.*, 2000; Satton *et al.*, 2001).

The differences in number of microorganisms were defined by the t-test at the 0.05 and 0.01 levels of probability.

Results and discussion

Number of microorganisms in rhizosphere of sunflower plants infected and non-infected by *O. cumana*

The processes taking place in the soil can be estimated by increasing the total number of microorganisms in the plant rhizosphere, since the active development of soil microorganisms indicates a high biological potential of the soil. Among the existing methods for assessing the biological activity of the soil in agrophytocenosis, the total number of microorganisms in the soil is considered to be the most complete. The ecological condition of the soil is characterized by qualitative and quantitative changes in the structure of the microbial coenosis and the ratio of the number of individual ecological and trophic groups of microorganisms reflecting the response to the action of various factors, including anthropogenic ones (Yeshchenko, 2011).

Analysis of the quantitative characteristics of microbial communities in the studied soils showed that the total number of bacteria in the rhizosphere of sunflower plants infected by broomrape did not differ significantly from control and was 11.7 and 12.1 million CFU / g of soil, respectively (Table 1).

The number of ammonifiers was quite low both in the rhizosphere of control and infected sunflower plants, which indicates a low content of organic substances in the soil. The number of bacterial microflora, using for its life mineral nitrogen (on SAA medium), in the rhizosphere of plants infected by *O. cumana* was also at the control level.

The number of both pedotrophs and oligotrophs in the compared soil samples did not differ, although in both cases the number of microorganisms of the latter group were significantly larger than the first. The increase in the number of oligotrophs indicates a decrease in nutrients necessary for the life of the soil microbiocenosis, which is due to their trophic specificity (oligotrophic microflora develops on poor soils) and lack of competition.

Both tested soil samples were characterized by a low content of bacteria of the genus *Azotobacter* (35% in the rhizosphere of uninfected plants and 21% in the rhizosphere of infected plants), which may be due to a rather acidic of the soil as well as low soil moisture content in the autumn period. However, the number of representatives of the *Azotobacter* genus in the rhizosphere of parasite-infected sunflower plants was significantly less than in control.

At the same time, the number of microscopic fungi growing both on the Czapek-Dox medium and on the SAA medium in the rhizosphere of sunflower infected by broomrape was almost 2 times higher than the number of these microorganisms in the rhizosphere of healthy plants. Obviously, an additional nutrient substrate appeared in the rhizosphere of plants infected by the parasite, creating a favorable environment for the development of this fungal microflora.

For a comprehensive assessment of microbiological processes in the studied soils, in addition to the number of individual ecological-trophic groups, the direction of microbiological processes was determined.

Analysis of the activity of microbiological processes in the rhizosphere of infected by the parasite and non-infected sunflower plants showed high, but close, mineralization-immobilization coefficients, indicating active decomposition

millionmillionmillionmillionCzapek-Dox starch-ammoniCzapek-Dox starch-ammoniagar mediuagar mediuagar mediuagar mediuagar mediuagar mediuagar mediuagar mediuagar mediubroomrapeB.03 ± 0.45 1.50 ± 0.15 0.73 ± 0.06 35 ± 4.1 34.2 ± 1.08 8.31 ± 0.16 broomrapeInfected by 1.59 ± 0.06 8.23 ± 0.51 1.76 ± 0.19 0.61 ± 0.05 $21 \pm 2.3^*$ $53.8 \pm 1.44^{**}$ 15.61 ± 0.82	Plants	Ammonifiers,	Bacteria utilizing mineral	Oligotrophs,	Pedotrophs,	Azotobacter, %	Fungi.	thousand
Non-infected by 1.67 ± 0.11 8.93 ± 0.45 1.50 ± 0.15 0.73 ± 0.06 35 ± 4.1 34.2 ± 1.08 8.31 ± 0.06 broomrape Infected by 1.59 ± 0.06 8.23 ± 0.51 1.76 ± 0.19 0.61 ± 0.05 $21 \pm 2.3^*$ $53.8 \pm 1.44^{**}$ 15.61 ± 0.82		million	nitrogen, million	million	million		Czapek-Dox medium	starch-ammonium agar medium
broom appendix to be a solution of the second second by 1.59 ± 0.06 solution 1.76 ± 0.19 or 61 ± 0.05 $21 \pm 2.3^{*}$ 53.8 $\pm 1.44^{**}$ 15.61 $\pm 0.82^{*}$	Non-infected by	1.67 ± 0.11	8.93 ± 0.45	1.50 ± 0.15	0.73 ± 0.06	35 ± 4.1	34.2 ± 1.08	8.31 ± 0.65
	broomrape Infected by	1.59 ± 0.06	8.23 ± 0.51	1.76 ± 0.19	0.61 ± 0.05	$21 \pm 2.3^*$	53.8 ± 1.44 **	$15.61 \pm 0.82^{**}$

Notes: *,** The differences are significant at the 0.05 and 0.01 levels of probability, respectively.

and mineralization of soil organic substances in both cases. The coefficients of oligotrophy, reflecting the degree of exhaustion in the soil of nutrients available to microorganisms, as well as the coefficients of pedotrophy, were similar (Table 2).

Table 2: Microbiological parameters of soil in the rhizosphere of sunflower infected and non-infected by *O. cumana*.

Plants	Microbiological coefficients			
	mineralization-immobilization	oligotrophy	pedotrophy	
Non-infected by broomrape	5,25	0,14	0,43	
Infected by broomrape	5,14	0,18	0,38	

Peculiarities of mycocenosis in rhizosphere of sunflower plants infected by *O. cumana*

As a result of studying the species diversity of soil fungi in the rhizosphere of sunflower plants infected and non-infected by *O. cumana*, we identified 45 morphological types of micromycetes belonging to *Zygomycota* (4) and *Deuteromycota* or anamorphic fungi (41). Of these, 26 species were identified.

The analysis of the species and generic composition of fungi showed that species belonging to the *Acremonium*, *Aspergillus*, *Cladosporium*, *Cephalosporium*, *Fusarium*, *Mucor*, *Paecilomyces*, *Penicillium*, *Rhizopus*, *Trihoderma*, *Verticillium* genera and *Mycelia sterilia* (white) were typical inhabitants of the investigated soils (Tables 3 and 4).

As can be seen from the Table 3, the *Aspergillus* (7 species) and *Penicillium* (7 species) genera were distinguished by the widest species diversity. The generic composition of the *Fusarium* genus and mucoral fungi was less diverse. The remaining genera of micromycetes were represented by 1–2 species. It should be noted a significant reduction in the species diversity of *Fusarium* genus and demacic fungi, which according to our data (Kostyuchenko and Lyakh, 2017) are typical species for the soils of Southern Steppe of Ukraine.

Mycocenosis, formed in the root zone of healthy sunflower plants, was characterized by the greatest species diversity (28 species), which exceeded almost 1.5 times the number of species in the rhizosphere of plants infected by the parasite.

An analysis of the similarity of the species composition of microscopic fungi in the investigated soils using the Sorensen index indicates that the similarity of micromycete complexes is at the level of 51% (Table 4).

Genus	Nu	umber of species, pcs.	(%)
	Total	Non-infected by broomrape	Infected by broomrape
Acremonium	1 (2.94)	1 (3.57)	-
Alternaria	1 (2.94)	1 (3.57)	-
Aspergillus	7 (20.59)	6 (21.43)	6 (31.58)
Cephalosporium	1 (2.94)	1 (3.57)	1 (5.26)
Fusarium	3 (8.82)	3 (10.71)	1 (5.26)
Mucor	3 (8.82)	2 (7.14)	2 (10.53)
Paecilomyces	2 (5.88)	2 (7.14)	-
Penicillium	7 (20.59)	6 (21.43)	4 (21.05)
Rhizoctonia	1 (2.94)	1 (3.57)	1 (5.26)
Rhizopus	1 (2.94)	-	1 (5.26)
Trihoderma	1 (2.94)	1(3.57)	-
Verticillium	2 (5.88)	2 (7.14)	-
Other species	4 (11.76)	2 (7.14)	3 (15.79)
Total species	34	28	19
Total genera	15	13	10

Table 3: The genus structure of micromycete complexes in the rhizosphere of sunflower plants infected and non-infected by *O. cumana*.

Table 4: The species structure of micromycete complexes in the rhizosphere of sunflower plants infected and non-infected by *O. cumana*.

	Species	Non-infected by broomrape	Infected by broomrape
	Zygomycota, Zygor	nycetes, Mucorales Mucoraceae	
1	Mucor hiemalis	+	_
2	M. racemosus	+	+
3	Mucor sp. 3	_	+
4	Rhizopus nigricans	-	+
	Hyphomycetes,	Hyphomycetales Moniliaceae	
5	Acremonium charticola var. subglutinans	+	-
6	Aspergillus alliaceus	+	+
7	A. candidus	+	+
8	A. melleus	+	+
9	A. niger	+	+
10	A. niveus	+	+

(continued)

Table 4: (continued)

	Species	Non-infected by broomrape	Infected by broomrape
11	A. ochraceus	-	+
12	A. ustus	+	-
13	Cephalosporium gramineum	+	+
14	Paecilomyces lilacinus	+	-
15	Paecilomyces sp.	+	-
16	Eupenicillium ochrosalmoneum	+	+
17	P. canescens	+	-
18	P. crustosum	+	-
19	P. nigricans	+	+
20	P. solitum	+	+
21	P. thomii	+	-
22	Penicillium sp. 1	-	+
23	Trichoderma viride	+	-
24	Verticillium album	+	-
25	Verticillium sp.	+	-
	Demo	atiaceae	
26	Alternaria helianthi	+	-
	Tuberculariales	, Tuberculariaceae	
27	Fusarium moniliforme var. lactis	+	-
28	F. moniliforme var. subglutinans	+	-
29	F. oxysporum var. orthoceras	+	+
	Agonomycetales	, Agonomycetaceae	
30	Rhizoctonia – Mycelia sterilia (white)	+	+
	Other species	2	3
	Total genera (species)	13(28)	10(19)
	Sorensen similarity coefficient	0,51	

12 species of micromycetes, such as Aspergillus alliaceus, A. candidus, A. melleus, A. niger, A. niveus, Cephalosporium gramineum, Fusarium oxysporum var. orthoceras, Mucor racemosus, Eupenicillium ochrosalmoneum, Penicillium nigricans, P. solitum, Rhizoctonia (Mycelia sterilia), are common species found in the rhizosphere of plants of both non-infected and infected by O. cumana.

Mycocenosis, formed in the rhizosphere of healthy sunflower plants, was distinguished by a variety of species of the *Aspergillus* (6) and *Penicillium* (6) genera. The proportion of these fungi was 42.9 % of all the derived fungi. However, it should be

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noted that the fungi of the Acremonium, Aspergillus (A. alliaceus, A. candidus), *Verticillium (V. albium)* genera, *Eupenicillium ochrosalmoneum* and *Mycelia sterilia* (white) dominated the soil under study. *Trichoderma viride* and *Paecilomyces lilacinus* species occurred only in the rhizosphere of non-infected plants (Figure 1(a)).



Figure 1: Typical micromycetes isolated from the rhizosphere of sunflower plants non-infected (A) and infected (B) by *O. cumana*.

A – 1 – Aspergillus alliaceus; 2 – Penicillium thomii; 3 – Verticillium sp.; 4 – Mycelia sterilia; 5 – Alternaria helianthi; 6 – Aspergillus niger; 7 – Aspergillus candidus; 8 – Aspergillus ustus; 9 – Paecilomyces lilacinus; 10 – Penicillium solitum.

B – 1 – Aspergillus alliaceus; 2 – Aspergillus melleus; 3 – Aspergillus niger; 4 – Aspergillus ochraceus; 5 – Rhizopus nigricans; 6 – Penicillium solitum.

In the rhizosphere of sunflower plants infected by broomrape, fungi of the *Aspergillus* genus, *Eupenicillium ochrosalmoneum*, *Penicillium nigricans*, *Mycelia sterilia* (white) and *Rhizopus nigricans* dominated (Figure 1(b)).

According to the experimental conditions, both non-infected and infected with broomrape sunflower plants were cultivated on an artificially created infectious background. Obviously, this was the reason for the restructuring in both cases of mycocenosis in the direction of the accumulation of resistant species of fungi that are able to compete actively in the conditions that have arisen. Such species include toxin-producing micromycetes of the Aspergillus and Penicillium genera with active conidiogenesis (Tables 3 and 4). In turn, the mycocenosis of plants infected by broomrape also undergoes changes in comparison with the mycocenosis of non-infected plants, further exacerbating the situation. Thus, in the rhizosphere of plants infected by the parasite, 10 species belonging to the Aspergillus and Penicillium genera were identified, which accounted for more than 50% of all identified species. In the rhizosphere of non-infected sunflower plants, the proportion of such fungi in the mycocenosis was significantly less. An increase in the proportion of toxin-forming fungi, and especially fungi of the Aspergillus genus, in the rhizosphere of plants infected with broomrape resulted in impoverishment of the generic and species composition of micromycetes (10 genera and 19 species - infected plants; 13 genera and 28 species – non-infected plants). It should be remembered that a decrease in the diversity of micromycetes in the rhizosphere of plants infected by broomrape compared with non-infected plants was accompanied by a significant increase in their numbers (Table 1).

The foregoing indicates that in the rhizosphere of sunflower plants infected by broomrape, a special mycocenosis is formed which is different from the mycocenosis of healthy plants. In favor of this conclusion are the data of Kirilova *et al.* (2018), who noted that parasitic plants can affect different groups of microorganisms due to a change in the chemical composition of the root exudates.

The possibility of broomrape to influence the microbial composition of the soil where it lives, may also suggest that this flower plant parasite has a special chemical composition. It should be noted that the useful properties of broomrape due to specific chemical composition have already been appreciated by folk healers for a long time. Moreover, it has been used both in Slavic and Eastern traditional medicine, where the crushed plant is recommended to be used for the treatment of wounds of various etiologies. Antimicrobial properties are described in Orobanche ramosa, Orobanche ammophyla and are obviously characteristic of other broomrapes (Makhlayuk, 1992).

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It is known that not only plants of the *Orobanche* genus, but also other members of the *Orobanchaceae* family, such as *Sistanche salsa*, for example, are used in traditional folk medicine. *Cistanche salsa* contains 5 times more biologically active compounds than ginseng and exhibits strong anti-bacterial and anti-viral effects (Hu and Feng, 2012).

Summarizing the results obtained, the following should be noted. In the conditions of the stationary infectious field of the Southern Steppe of Ukraine, the rhizosphere's soil of the sunflower plants non-infected and infected by broomrape did not differ in the number of bacteria of ammonifiers, bacteria using mineral nitrogen, oligotrophs and pedotrophs and only revealed a decrease in the *Azotobacter* quantity in the rhizosphere of diseased plants. At the same time, in the latter case, a significant increase in the number of microscopic fungi was detected. The structure of the fungal cenosis also underwent significant changes, which were expressed both in the reduction of the generic and species diversity, and in the increase in the proportion of toxin-forming fungi of the *Aspergillus* and *Penicillium* genera, characterized by active conidiogenesis.

References

- Antonova, T.S., Araslanova, N.M., Strelnikov, E.A., Ramazanova, S.A., Guchetl, S.Z., Tchelyustnikova, T.A., 2012. Some peculiarities of ontogenesis of *Orobanche cumana* Wallr., parasitizing on sunflower in Rostov region of Russian Federation. Helia 35(56): 99–110.
- Babayants, O.V., 2011. Immunological characteristic of wheat plant resources and verification of genetic protection against pathogens of fungal aetiology in the Steppe region of Ukraine: Thesis for research degree of Doctor of Sciences in Biology on speciality 06.01.11 Phytopatology, the National University of Life and Environmental Sciences of Ukraine, Kyiv, pp. 48 (In Ukrainian).
- Badri, D.V., Vivanco, J.M., 2009. Regulation and function of root exudates. Plant, Cell & Environment 32(6): 666–681.
- Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in Rhizosphere interactions with plants and other organisms. Annual Review of Plant Biology 57: 233–266.
- Belyuchenko, I.S., 2016. Micromycete composition in pure and combined crops in cultivated land. Biotechnology Research Asia 13(3): 1567–1572.

Bilaj, V.I., Koval, E.Z., 1988. Aspergillus, Nauk, Dumka, Kiev, pp. 204. (In Russian).

- Domsh, K.H., Gams, W., Andersen, T.H., 1993. Compendium of Soil Fungi, Acad. Press, London, pp. 859.
- Dudka, I.A., Vasser, S.P., Ellanskaya, I.A., et al., 1982. Methods of Experimental Mycology, Reference book (ed. V.I. Bilaj). Nauk, Dumka, Kiev, pp. 550. (In Russian).
- Fernandez-Martinez, J.M., Dominguez, J., Perez-Vich, B., Velasco, L., 2008. Update on breeding for resistance to sunflower broomrape. Helia 31: 73–84.

- Haldar, S., Sengupta, S., 2015. Plant-microbe cross-talk in the Rhizosphere: Insight and biotechnological potential. Open Microbiology Journal 9: 1–7.
- Hoog, G.S., Guarro, J., Gene, J., Figueras, M.J., 2000. Atlas of Clinical Fungi, Univ. Rovira i Virgili, Reus, pp. 1126.
- Hu, J.Q., Feng, J.Y., 2012. The chemical composition and pharmacological effects of *Cistanche*. Clinical Journal of Chinese Medicine 15: 26–28.
- Kaya, Y., Evci, G., Pekcan, V., Gucer, T., 2004. Determining new broomrape infested areas, resistant lines and hybrids in Trakya region of Turkey. Helia 27: 211–218.
- Kaya, Y., 2014. Current situation of sunflower broomrape around the world. *In*: Proceedings of 3rd International Symposium on Broomrape (*Orobanche* spp.) in Sunflower, Cordoba, Spain, pp. 9–18.
- Kirilova, I., Hristeva, T., Bozhinova, R., Mishev, K., Vassileva, V., Dimitrova, A., Denev, I., 2018. Plant parasitism from the prospective of the system host plants - parasitic broomrapes-soil microbiome. In: Proceedings International Congress on Oil and Proteins Crops. Meeting of the EUCARPIA Oil and Protein Crops Section, Chisinau, Republica Moldova, pp. 108.
- Kizilkaya, R., 2009. Nitrogen fixation capacity of Azotobacter spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. Journal Environment Biology 30(1): 73–82.
- Kostyuchenko, N.I., Lyakh, V.A., 2017. Peculiarities of taxonomic structure of micromycete complex in root zone of sunflower in conditions of Southern Steppe of Ukraine. Helia 40 (67): 147–159.
- Kurdish, I.V., 2010. Introductions of Microorganisms in Agricultural System, Naukova Dumka, Kiev, pp. 255. (In Ukrainian).
- Mahaffee, W.F., Klopper, J.W., 1997. Temporal changes in the bacterial communities of soil, rhizosphere and endorhiza associated with field-grown cucumder (*Cucumis sativus* L.). Microbiology Ecology 34: 210–233.

Makhlayuk, V.P., 1992. Medicinal Plants in Folk Medicine, Niva Rossii, Moscow, pp. 477. (In Russian).

- Megarran, E., 1992. Ecological Diversity and Its Measurement, Mir, Moscow, pp. 184. (In Russian).
- Melero-Vara, J.M., Dominguez, J., Fernandez-Martinez, J.M., 2000. Update on sunflower broomrape situation in Spain: Racial status and sunflower breeding for resistance. Helia 23(33): 45–55.
- Mirchink, T.G., 1988. Soil Mycology, MGU, Moscow, pp. 220. (In Russian).
- Pacureanu-Joita, M., Ciuca, M., Sava, E., 2012. Broomrape (Orobanche cumana Wallr.), the most important parasite in sunflower: Virulence, race distribution, host resistance. In: Proceedings of 18th International Sunflower Conference, Mar del Plata, Argentina, pp. 1052–1057.
- Satton, D., Fothergill, A., Rinaldi, M., 2001. Guide to Pathogenic and Opportunistic Fungi, Mir, Moscow, pp. 486. (In Russian).
- Shindrova, P., 2006. Broomrape (*Orobanche cumana* Wallr.) in Bulgaria-distribution and race composition. Helia 29(44): 111–120.
- Yang, C.-H., Crowley, D.E., 2000. Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. Applied and Environment Microbiology 66(1): 345–451.
- Yeshchenko, V.O., 2011. To the method of determining the biological activity of the soil. Collection of Scientific Works of Uman National University of Horticulture 77: 21–26. (In Ukrainian).
- Zvyagintsev, D.G., 1991. Methods of Soil Microbiology and Biochemistry, MGU, Moscow, pp. 303. (In Russian).

DE GRUYTER

Résumé

Broomrape (*Orobanche cumana* Wallr.) peut influencer la cénose microbienne dans la rhizosphère du tournesol

Les complexes bactériens et micromycètes de la rhizosphère des plants de tournesol non infectés et infectés par le broomrape (Orobanche cumana Wallr.) ont été comparées. Les investigations ont été menées dans les conditions d'un champ infectieux stationnaire enrichi annuellement en résidus de plantes infectés et en graines de broomrape collectées dans différentes régions de l'Ukraine. Le sol est lessivé, le chernozem à faible humidité avec un pH acide. Les échantillons de sol sélectionnés à la fin de la végétation de la rhizosphère des plantes saines et infectées par le broomrape des échantillons de reproduction de tournesol. Le nombre total de bactéries trouvées dans la rhizosphère des plants de tournesol infectés par le parasite ne différait pas significativement de celui des témoins et était respectivement de 11,7 et 12,1 millions d'UFC / g de sol. Le nombre d'ammonificateurs ainsi que la microflore bactérienne, utilisant pour sa vie l'azote minéral, et les pédotrophes et oligotrophes dans les échantillons de sol comparés, ne différaient pas de manière significative et correspondaient généralement à ce type de sol. Bien qu'en général les deux échantillons de sol testés aient été caractérisés par une faible teneur en bactéries du genre Azotobacter, le nombre de représentants de ce genre dans la rhizosphère des plantes infectées par le parasite était légèrement inférieur à celui des témoins (35 % et 21 %, respectivement). Cependant, contrairement à la plupart des bactéries, le nombre de micromycètes détectés sur les milieux Czapek-Dox et gélose-amidon-ammoniac, dans la rhizosphère des plantes infectées par le broomrape, a presque doublé le nombre de ces microorganismes dans la rhizosphère des plantes saines. L'analyse de la composition générique et de la composition spécifique de hongos microscopiques a montré que dans la rhizosphère des plants de tournesol infectés par le parasite, une mycocénose très spécifique diffère d'une mycocénose de plantes saines. Cette mycocénose était caractérisée par un nombre beaucoup plus faible de genres et d'espèces de micromycètes. Dans le même temps, pour la structure de la cénose fongique des plantes malades, il y avait une augmentation caractéristique de la proportion de hongos formant des toxines des genres Aspergillus et Penicillium avec une conidiogenèse active. Les données obtenues témoignent non seulement des différences de complexes microbiens dans la rhizosphère des plants de tournesol non infectés et infectés par le broomrape, mais indiquent également la direction d'action de ce parasite.

Resumen

Broomrape (*Orobanche cumana* Wallr.) puede influir en el cenosis microbiano en la rizosfera del girasol

Se han comparado los complejos bacterianos y micromicéticos en la rizosfera de plantas de girasol no infectadas e infectadas con broomrape (Orobanche cumana Wallr.). Las investigaciones se llevaron a cabo en las condiciones de un campo infeccioso estacionario que se enriqueció anualmente con residuos vegetales infectados y semillas de broomrape recogidas en diferentes regiones de Ucrania. El suelo es chernozem liofilizado con pH ácido. Las muestras de suelo seleccionadas al final de la vegetación de la rizosfera de las plantas sanas e infectadas con broomrape de girasol. El número total de bacterias encontradas en la rizosfera de plantas de girasol infectadas por el parásito no difirió significativamente del control y fue de 11.7 y 12.1 millones de UFC / g de suelo, respectivamente. El número de amonificantes así como también de microflora bacteriana, que utiliza nitrógeno mineral durante su vida útil, y pedotrofos y oligotrofos en las muestras de suelo comparadas no difirió significativamente, y generalmente correspondía a este tipo de suelo. Aunque en general, las dos muestras de suelo evaluadas se caracterizaron por un bajo contenido de bacterias del género Azotobacter, el número de representantes de este género en la rizosfera de plantas infectadas con parásitos fue algo menor que el control (35 % y 21 %, respectivamente). Sin embargo, a diferencia de la mayoría de las bacterias, la cantidad de micromicetos detectados en los medios de agar Czapek-Dox y almidón-amoníaco, en la rizosfera de las plantas infectadas con broomrape, casi excedió el número de estos microorganismos en la rizosfera de las plantas sanas. El análisis de la composición genérica y de especies de hongos microscópicos mostró que en la rizosfera de las plantas de girasol infectadas por el parásito se formó una micocenosis muy específica que difiere de una micocenosis de plantas sanas. Esta mycocenosis se caracterizó por un número mucho más pequeño de géneros y especies de micromicetos. Al mismo tiempo, para la estructura de la cenosis fúngica de plantas enfermas, hubo un aumento característico en la proporción de hongos formadores de toxinas de los géneros Aspergillus y Penicillium con conidiogénesis activa. Los datos obtenidos testifican no solo las diferencias en los complejos microbianos en la rizosfera de las plantas de girasol no infectadas e infectadas por broomrape, sino que también indican la dirección de acción de este parásito.