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Cenosis structure and species composition dynamics of micromycetes decomposing crop root residues in soil

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Experiments on the decomposition of crop residues were carried out at the Experimental Station of the Lithuanian University of Agriculture (54°53'N, 23°50'E) in a model field in 2004–2006. The soil of the experimental site is *Endocalcari-Epihypogleyic Cambisol* (*sicco*) (CMg-p-w-can).

We investigated root residues of winter oilseed rape (*Brassica napus* L. ssp. *oleifera biennis* Metzg.), spring oilseed rape (*Brassica napus* L. ssp. *oleifera annua* Metzg.), winter wheat (*Triticum aestivum* L.), and red clover (*Trifolium pratense* L.) decomposed in soil for different time (7.5, 14.5, 19.5, and 26.5 months). The aim of the investigation was to establish in soil decomposed winter and spring rape root micromycetes community structure and species composition dynamics in comparison with winter wheat and clover roots.

It has been established that on the decomposed crop roots micromycetes spread depended first of all on the plant species. Most of micromycetes colony forming units were found on red clover roots, less on rape roots and least on winter wheat roots. The micromycetes colony forming unit number depended on root decomposition (according to correlative-regressive analysis a positive strong and very strong correlation) and on the decomposed substrate C : N ratio (a strong and very strong negative correlation). A specific character of the genus *Trichoderma* fungi species spreading depending on the nature of crop roots has been established. For the first time in Lithuania the following species have been identified: *T. longibrachiatum* – on winter and spring rape, *T. aggressivum f. europaeum* – on winter rape, *T. spirale* – on spring rape, winter wheat and clover, *T. crassum* – on winter wheat and winter rape, *T. brevicompactum* – on winter wheat roots. On the decomposed rape roots *Trichoderma* micromycetes producing antibiotics were prevailing (*T. harzianum, T. aggressivum f. europaeum, T. viride, T. hamatum and others*) and limited the development of conditionally phytopathogenic *Fusarium, Rhizoctonia, Penicillium, Alternaria* and other fungi. In all periods of study, on rape roots the number of *Fusarium* species was lower than on clover or wheat roots. Thus, *Trichoderma* fungi abundance on rape residues, rape root chemical composition and the peculiarities of their fragmentation products are important factors influencing the phytosanitary state of soil.

Key words: crop root residues, decomposition, micromycetes, carbon and nitrogen ratio

INTRODUCTION

Accumulation of organic substances in the upper horizon of soil is a complicated biochemical process in which soil biota determines the course of substance metabolism and the stability of an ecosystem. In every system, biota individuals are related by close trophic ties [1]. Soil animals comminute crop residues, thus increasing their area and creating suitable conditions for the activity of microorganisms. In the destruction of crop residues microorganisms of different groups participate, but fungi are the principal decomposers of cellulose, hemicellulose, lignin, pectin. When perishing, the soil biota every year leaves on average 100–200 kg ha^{-1} of organic substances [2]. The principal function of microorganisms in soil is mineralization and humification of crop residues. The course of these processes is determined by climate, the composition of a soil-forming rock, the type of phytocenosis, the systematic units of soil, its acidity, moisture, oxygen content, fauna structure and other factors [3]. The highest variety of microorganisms has been established in Middle Lithuanian lowland RDg (*Cambisols*) and IDg (*Luvisols*) soils. In it, micromycetes of the genus *Penicillium* are spread less, while *Mucor, Trichoderma, Fusarium* fungi are found more often. It is known that *Mucor* and *Trichoderma* micromycetes prefer better cultivated soils rich in organic matter, whereas *Fusarium* fungi like grass plantations [4]. For the functioning of micromycetes of every sort, a suitable nutrition

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substratum and environment conditions are necessary. These conditions are largely influenced by plant residues of various chemical composition and by the tilling of land [5]. In Middle Lithuanian *Endocalcari-Epihypogleyic Cambisol* soils the residues of plants (chopped straw) increase the population of micromycetes by 24–40% and the traditional deep ploughing by 11% [6]. However, mineralization-humification processes in the soil depend not so much on the quantity of micromycetes than on the physiological properties of their species. Micromycetes are viable organisms noted for good adaptation properties, which participate in various energy metabolism processes – beginning with complex decomposition of chemical compounds to the biosynthesis of new biological substances [7, 8]. The economic character of micromycetes nutrients metabolism is exclusive – 60% of decomposed carbon and nitrogen are immobilized in the very organisms, by this temporarily keeping the nutrients in the soil [9]. Micromycetes participate in all intermediate processes of plant residue decomposition, they decompose cellulose up to glucose and citric acid, proteins to amino acids, ammonia, organic acids and lignin to phenol alcohols and aldehydes [10–12].

Soil biology investigations are extremely important and topical as the anthropogenic effect on agrocenoses increases and plants of a high biopotential are grown. In Lithuania, in this sphere of science the works of A. Lugauskas, I. Eitminavičiūtė, M. Eidukevičienė, E. Lapinskas and other scientists are widely known. However, there are no data on winter and spring rape which are more and more widely grown for alimentary oil and biofuel. The effect of rape on humus stability and soil fertility holds out for two years [13].

The aim of our investigation was to establish the structure of micromycetes cenosis of winter and spring rape root residues decomposed in the soil, the dynamics of their species composition in comparison with winter wheat and clover roots which have been more investigated in this respect.

MATERIALS AND METHODS

Experiments of decomposition of crop residues were carried out at the Experimental Station of the Lithuanian University of Agriculture (54°53'N, 23°50'E) in a model field experiment during the period 2004–2006. The soil of the experimental site, according to the classification of the year 1999 (LTDK-99), is *Endocalcari-Epihypogleyic Cambisol* (*sicco*) (CMg-p-w-can). Soil pH 6.86–6.92, humus content in the arable layer 2.35–2.43%, total N 1.30–1.47 g kg^{-1}, base saturation >90%, available phosphorus (P_2O_5) 158–255 mg kg^{-1}, available potassium (K_2O) 124–167 mg kg^{-1}, available sulphur (SO_4^{2-}) 12.3–18.6 mg kg^{-1}. In the granulometric composition of the soil profile arable layer (0–20) prevail silt (0.05–0.002 mm) – 55.3% and sand (2.00–0.05 mm) – 33.8%, while clay particles (<0.002 mm) amount only to 10.9%; $C_{org.}$: N = 9.2. The layer under the arable horizon (32–42 cm) is dominated by clay particles (38.6%), and $C_{org.}$: N was highest among all horizons of the soil profile (10.7). In the deeper than 50 cm layers clay particles amount to 43.7–68.0%, and $C_{org.}$: N decreases with deepness from 7.6 to 5.7.

The experiment had a two-factor design: factor A – crop root residues: 1. Roots of winter oilseed rape (*Brassica napus* L. ssp. *oleifera biennis* Metzg.); 2. Roots of spring oilseed rape (*Brassica napus* L. ssp. *oleifera annua* Metzg.); 3. Roots of winter wheat (*Triticum aestivum* L.); 4. Roots of red clover (*Trifolium pratense* L.); factor B – decomposition duration (1. 7.5; 2. 14.5; 3. 19.5; 4. 26.5 months).

The experiment started on 1 September 2004. The initiation and end datum-point of each study period (except the initial stage) set up when the average temperature in a 20 cm soil depth for three successive days in spring was ≥ 5 °C and in autumn ≤ 5 °C. Samples of rape and wheat roots were prepared after harvesting. The sampling of roots of second-year red clover was done after the first grass cut. Crop roots were collected from the arable layer, they were cleaned and chopped in 2–3 cm long chaffs. The content of dry matter in them was estimated. Samples of natural humidity and 20 g weight were taken and put into 9 × 12 cm net polychlorvinyl bags with 0.05 mm mesh diameter. Bags with crop residues were incorporated in ploughed up furrow of black fallow at the depth 20 cm, with 20 cm spaces. A particular part of bags with crop root residues at the end of research periods were dug out and cleaned from soil. At the Laboratory of Biodeterioration Research of Institute of Botany, the number of micromycetes colony forming units on crop root residues was estimated and identification of isolates was performed. The chemical composition of crop root residues was established at the Experimental Station and TEMPUS laboratory of the Lithuanian University of Agriculture.

For the micromycetes evolution, the ablution and direct dissemination methods and for the nutritive medium agar beer mash (6°Bal.) were used. For isolation of pure cultures, the Chapeck and potato-dextrose agar medium were used. The number of micromycetes on crop root residues was calculated according to the formula:

$$a = b \times c \times d / e,$$

where a is the number of micromycetes colony-forming units, cfu (g^{-1} dry matter, dm); b is the volume of disseminated suspension, ml; c is the number of developed colonies; d – dilution of suspension; e – the mass of dry substrate, g [14].

The species of micromycetes were identified according to cultural and morphological features using describers [15–25].

The content of a bag was dried out until air-dry weight, grounded, sieved through a 1 mm separator. The following chemical analyses of samples were performed: dry matter content determined by drying in a thermostat at 105 °C, the content of total nitrogen by the Kjeldahl and organic carbon by the Tyurin methods.

Soil agrochemical and physical analysis methods: granulometric composition – the pipette method according to FAO/ISRIC, pH_{KCl} – potentiometric, organic carbon – the Walkley–Black, total N – the Kjeldahl and humus – the Tyurin methods, base saturation was calculated from the sum of absorbed bases (Kappen–Hilkovitz method), mobile P_2O_5, K_2O, SO_4^{2-} was determined with an infrared ray PSCO/ISI IBM-PC 4250 spectrophotometer according to the data bank designed in conformity with data of analyses performed by the reference methods [26].

Statistical analysis of the data was performed using Excel 98, ANOVA for two-factor experiment and STAT for correlation analysis from SELEKCIJA package [27].

Investigations were carried out during cold and warm periods (Table 1).

The average weather temperature of both cold periods was negative, in soil at the depth of 20 cm positive, but in the first period the temperature was by 1.0 °C and soil moisture by 5.1 percent units higher than in the second period.

In the warm period of 2006, the air temperature was by ~2.0 °C and of soil at the depth of 20 cm by ~1.0 °C higher and precipitation by 77.4 mm more than during the same period of 2005. The average air temperature for three successive days before digging out the samples differed by ~1.0 °C, and in the soil at the depth of 20 cm the average temperature differed by 1.5 °C. At the end of the warm period of 2005, soil moisture was by 4.6 percent units higher than at the end of the same period of 2006. For the warm periods, a sharp shift of prolonged drought, high air temperatures and rainfall is characteristic.

RESULTS

The amount of micromycetes colony forming units on the crop root residues decomposed in the soil was very different (Table 2). After 7.5 months of crop root decomposition, samples in spring were dug from the depth of 20 cm with soil moisture 26.8% and the average temperature for 3 days 6.5 °C (Table 1). After this cold period (average soil temperature 1.4 °C) and minimal microorganism activity, an intensive crop root decomposition started, especially on the substrate suitable for micromycetes – red clover roots of which 33% were decomposed and the carbon and nitrogen ratio (17.2) was favourable for mineralization (Figs. 1, 2). When the investigation began, in the winter rape roots the highest carbon and nitrogen ratio (105.5) was established, which after the first investigation period reduced to 46.0 (Fig. 1). After 7.5 months of root decomposition, the largest quantity of micromycetes colony forming units was found in red clover roots, less on rape roots and the least on winter wheat roots (Table 2). For the spread of micromycetes, decomposing winter but not spring rape roots were more suitable. Such different spreading of micromycetes on the roots of various decomposed crops was typical of all the study periods.

In autumn, after 14.5 months when in the soil depth of 20 cm where the root decomposition took place the temperature fell to 3.2 °C, the number of micromycetes colony forming units on crop roots decreased: on clover by 22.7%, on winter wheat by 41.4% and on rape by 47%. However, with a very intensive microorganisms' activity in this warm period, crop root decomposition was more intensive: clover – 2.2 times, spring rape – 1.9 times, winter rape – 1.6 times, winter wheat – 1.2 times in comparison with the earlier phase of the study.

The second cold period (winter of 2005/2006) was colder and dryer than the first. At the depth of 20 cm the average soil temperature was 0.5 °C. In spring, during the digging of the

Table 1. **Duration of investigation periods and their meteorological conditions**
LUA Experimental Station, 2004–2006

No.	Duration of investigation period (months from initiation)	Date	During period			Average conditions for successive 3 days before resurrection of samples		
			average temperature (°C)		precipitation (mm)	temperature (°C)		soil moisture at a depth of 20 cm (%)
			air	soil at a depth of 20 cm		air	soil at a depth of 20 cm	
1.	7.5	11 11 2004–11 04 2005	–1.1	1.4	216.0	6.7	6.5	26.8
2.	14.5	11 04 2005–02 11 2005	13.4	15.0	399.0	2.3	3.2	21.6
3.	19.5	02 11 2005–24 04 2006	–2.0	0.5	157.0	8.1	7.5	21.7
4.	26.5	24 04 2006–02 11 2006	15.1	16.1	476.4	3.5	4.7	17.0

Table 2. **Spread of micromycetes on crop root residues decomposing in soil (cfu × 10^3 g^{-1} d. m.)**
LUA Experimental Station, 2004–2006

Crop root residue	Duration of crop root residue decomposition in soil (months)			
	7.5	14.5	19.5	26.5
Winter rape	494.4 ± 24.3	261.7 ± 13.8	688.4 ± 41.8	750.4 ± 40.4
Spring rape	442.8 ± 17.1	234.9 ± 10.1	757.4 ± 41.1	443.1 ± 18.3
Winter wheat	317.7 ± 13.0	186.3 ± 9.3	138.2 ± 18.5	288.1 ± 9.8
Red clover	759.7 ± 33.1	587.8 ± 18.9	1101.3 ± 152.4	5097.6 ± 152.4

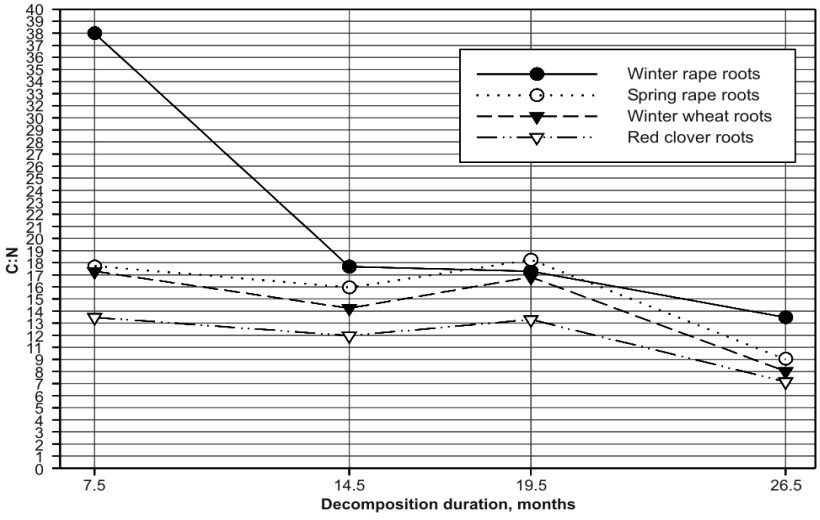

Fig. 1. C : N ratio in crop root residues incorporated into soil. $LSD_{05} = 1.05$

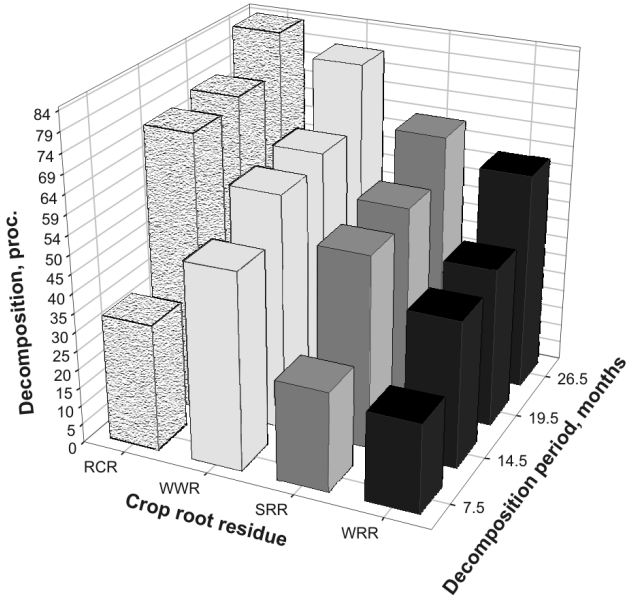

Fig. 2. Decomposition of crop root residues incorporated into soil. WRR – winter rape roots, SRR – spring rape roots, WWR – winter wheat roots, RCR – red clover roots. $LSD_{05} = 4.95$

samples (after 19.5 months of decomposition) at the depth of 20 cm the soil moisture was 21.7% and the average 3-day temperature 7.5 °C. Micromycetes colony forming units were more numerous by 29–42% on rape and clover roots and by 57% less on winter wheat roots than after the first cold period.

In that period, most numerous micromycetes colony forming units were found on clover (C : N ratio 8.4) and winter rape roots (C : N ratio 13.9). The least number of micromycetes colony forming units was found on winter wheat roots after 19.5 months of decomposition and the largest number – on spring rape roots when they were destroyed by 53.3% and the ratio of carbon and nitrogen from the initial level decreased by 73.8%.

A correlative-regressive analysis of the cold study period data revealed a very strong statistically significant positive correlation between the number of micromycetes colony forming units and the decomposition of winter rape roots ($r = 0.92$; $P \leq 0.01$). The micromycetes spreading dependence on the carbon and nitrogen ratio in decomposed winter rape roots was strong negative ($r = -0.83$; $P \leq 0.01$) and in spring rape, winter wheat and red clover roots very strong negative ($r = -0.93–0.98$; $P \leq 0.01$).

The second warm period (2006) was by 1.7 °C warmer and wetter (by 77.4 mm more precipitation) than the first one (2005). At taking samples in the autumn, at the depth of 20 cm the soil moisture was 17.0% and the average 3-day temperature 4.7 °C. At the end of the study, after 26.5 months of crop root decomposition, clover roots were most strongly decomposed (84.4%), their C : N ratio being 7.5. On these residues, the largest number of micromycetes were found – 4.6 times more than during the earlier period. The number of micromycetes colony forming units on the decomposed winter wheat roots increased twice (decomposition was 79%), on winter rape roots by 9% and their decomposition was only 57%. In the warm periods, the dependence of micromycetes colony forming units on the winter rape root decomposition was very strong and statistically significant positive ($r = 0.98$, $P \leq 0.01$). The correlation between the micromycetes colony forming units and the carbon and nitrogen ratio of decomposed crop root substrate was negative and very strong in winter rape ($r = -0.99$, $P \leq 0.01$), spring rape ($r = -0.97$; $P \leq 0.01$), red clover ($r = -0.96$, $P \leq 0.01$) and weak negative in winter wheat ($r = -0.48$, $P \leq 0.05$).

From clover, winter wheat, winter and spring rape roots decomposed in the soil, micromycetes belonging to 47 genera were revealed and identified (Table 3). Micromycetes species from 18 genera can be attributed to incidental, as their isolates were found only once. At the beginning of investigation (after 7.5 months of crop residue decomposition), in spring, on crop root residues an abundant variety of micromycetes genera and species was established, which in autumn (after 14.5 months) markedly decreased. Red clover roots were first of all colonized by the rapidly growing, sugar lysing *Mucorales* (*Mucor* and *Rhizopus* genera) fungi, also *Penicillium, Mortierella, Acremonium, Verticillium* and other fungi were isolated. Conditionally phytopathogenic *Fusarium* micromycetes were abundantly spread; they obviously penetrated into clover roots and survived after their perishing. In the second spring

Table 3. **Variability and spread of micromycetes species on decomposing crop root residues**
LUA Experimental Station, 2005–2006

Micromycetes genus	Duration of crop root residue decomposition in soil (months)															
	7.5				14.5				19.5				26.5			
	Number of micromycetes species															
	RC*	WW*	WR*	SR*	RC	WW	WR	SR	RC	WW	WR	SR	RC	WW	WR	SR
Acremonium	5	1	1	1		4	4	3	2	2	1	3		1	2	2
Alternaria									1				1	1		
Aureobasidium							1		1		1				1	
Botryotrichum				1	1			1				1				1
Cylindrocarpon					1				1							
Chaetomium									1			2				
Choenephora								1				1				
Cladosporium		2		2		2	1	2	1							
Curvularia				1				1								
Epicoccum	1		1													
Fusarium	6	7	4	3	7	10	4	4	6	6	3	3	8	4	3	4
Geotrichum						2	1			2						
Gliocladium	1			1										1		
Humicola		1	1	1						1	1	1		1		1
Mycelia sterilia	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Myceliophthora							1		1							
Myrothecium		1												2	4	1
Mortierella	5	3	5	2					3	3	1	3	1	2	4	1
Mucor	2	2	7	7		1	3	1	1	3		1	2	2	1	2
Oidiodendron	1								2	1				1	1	1
Paecilomyces	1								1			1		3		1
Penicillium	13	15	14	16	2	4	1	1	4			3	4	4		5
Rhizomucor		1			1				1				1			2
Rhizopus	2	2	1	1	2	1	1	2				1	1	2		1
Sclerotinia	1	1	1	1		1		1		1						
Sporotrichum	1			1				1	1	2						
Talaromyces	1					1										
Trichoderma	1	4	3	4		2	4	3	3	3	3	5	1	3	4	3
Verticillium	2	1		1						1			2	1	2	2
Other**	2		2			3	1	1	6	1				2		
All genera	18	14	13	16	7	14	12	14	23	13	7	13	10	17	10	15
All species	46	42	41	44	15	32	23	23	37	27	11	26	22	31	23	28

* RC – red clover; WW – winter wheat; WR – winter rape; SR – spring rape.

** Occasional species (once isolated) of the genus: *Absidia, Aspergillus, Bipolaris, Chrysosporium, Doratomyces, Drechslera, Hemicorynespora, Rhizoctonia, Scytalidium, Scopulariopsis, Sepedonium, Stachybotrys, Trichosporiella, Trichurus, Ulocladium, Volutella, Wardomyces, Zygorhynchus.*

(after 19.5 months of crop residue decomposition), on clover roots the biggest number of micromycetes species (37) was established. However, incidental species were prevailing and increased the number of the genus *Trichoderma* fungi which are able to assimilate both easily accessible carbohydrates (sugar, starch, hemicellulose) and cellulose, lignin, chitin and other organic substances, to decompose insoluble phosphates, pesticides [22]. Micromycetes of this genus actively participate in soil microorganism association, as they are characterized by a rapid growth, antagonistic properties in respect of other microorganisms, produce many antibiotic substances and toxins of which more than 40 are known [28]. *Trichoderma*

harzianum Rifai, *T. hamatum* (Bonord.) Bainier, *T. spirale* Bissett species fungi revealed after 19.5 months, were spread both on winter wheat and on spring rape roots. On winter rape roots, besides *T. harzianum* and *T. hamatum* species, also *T. aggressivum f. europaeum* Samuels et W. Gams species fungi were found. *T. polysporum* (Link ex Fr.) Rifai isolates were revealed only in clover and spring rape roots.

On winter rape roots, after the first investigation period, in comparison with the other crop roots, one of the lowest varieties of micromycetes was been established, which most markedly (nearly 2 times) decreased after the second spring (after 19.5 months). However, typical species destroying cellulose and lignin were isolated from the genera *Trichoderma* and *Fusarium*: *Trichoderma hamatum, T. harzianum* and *T. aggressivum f. europaeum*, *Fusarium oxysporum* Schldl., *F. moniliforme* J. Sheld., *F. heterosporum* Nees species. In the autumn of the last study period (after 26.5 months), on the decomposed clover roots only species of 10 genera were left (more than 2 times less than in spring after 19.5 months). After disappearance of many incidental species *Trichoderma polysporum* (Link ex Pers.) Rifai remained, the *Fusarium, Mucor* and *Verticillium* species variety slightly increased, whereas on other crop roots both the micromycetes genera and their species were more abundant and diverse than in spring (after 19.5 months).

After the first study period (after 7.5 months of decomposition), among micromycetes according to the number of species, fungi of the genus *Penicillium* prevailed. On all the roots of plants, active producers of secondary metabolites were established (*Penicillium expansum* Link, *P. ochro-chloron* Biourge and *P. fellutanum* Biourge). On clover and winter wheat roots the following *Penicillium* species were established: *P. rugulosum* Thom, which is an active cellulose and lignin degrader [3], and *P. diversum* Raper et Fennell, *P. albicans* Bainier, *P. chermesinum* Biourge, *P. griseo-fulvum* Dierckx. These species were not found on rape roots, however, from them *P. cyaneum* (Bainier et Sartory) Biourge species were isolated, which were not found on clover and winter wheat roots. *P. cyaneum* fungi usually develop on substrates rich in cellulose and lignin. *P. capsulatum* Raper et Fennell was isolated from rape roots; it widely spreads on plant residues, polymer substrates and easily adapts to various conditions of the environment, *P. clavigerum* Demelius belonging to the group of fungi degraders producing the enzyme laccase and decomposing substances containing phenol compounds [3]. In all the next experiments, *Penicillium* species were on average thrice as rare as at the beginning of the experiment. On winter rape roots during the last study (after 19.5 and 26.5 months), *Penicillium* micromycetes were not found at all.

Fusarium micromycetes should be noted for a rather abundant variety of species, but less than that of *Penicillium* during different crop root decomposition periods. *F. oxysporum* and *F. moniliforme,* isolating physiologically active enzymes *F. heterosporum, F. solini* (Mart.) Appel et Wollenw., *F. poae* (Peck) Wollenw., and at the end of experiments *F. avenaceum* (Fr.) Sacc. were most frequently spread on the roots of all plants and produced strong antibiotics and toxins. Half as many *Fusarium* species were found on the decomposing rape roots in all the periods of experiments as compared with clover and wheat roots.

Constant components of the organic compound decomposers' association are *Acremonium* micromycetes. Fungi of this genus were found on crop roots in all the study periods, except clover root residues in which they were found only in spring (after 7.5 and 19.5 months). Most abundant were *A. strigtum* W. Gams, *A. roseum* Petch, *A. charticola* (Lindau) W. Gams. These micromycetes should be noted for high cellulolytic and pectinolytic activity. The highest *Acremonium* species variety was found on decomposing rape roots after 14.5 months of decomposition. On the residues of winter rape roots, in this period only the typical species *A. kiliense* Grűtz and *A. potronii* Vuill. were identified, and on spring rape roots *A. cerealis* (P. Karst.) W. Gams, *A. furcatum* (Moreau et R. Moreau) ex W. Gams, *A. fusidioides* (Nicot) W. Gams were found.

On crop roots, typical species of soil saprophytes from the *Mortierella, Mucor, Rhizopus* genera were found. Fungi of separate species of these genuses are able to decompose starch, sugars, cellulose, chitin and other polymers. The species composition of these genera on the roots of plants mostly depended on the degree of root degradation. The highest variety of the genus *Mucor* species (*M. luteus* Linnem., *M. murorum* Naumov, *M. hiemalis* Wehmer, *M. racemosus* Fresen., *M. globosus* A. Fisch., *M. circinelloides* Tiegh., *M. plumbeus* Bonord.) was established on winter and spring rape after 7.5 months of decomposition. During the next decomposition periods when the above-mentioned species disappeared, *M. luteus*, *M. hiemalis* and *M. racemosus* species fungi remained; they were also spread on dissociated clover and winter wheat roots. In the first study period (after 7.5 months), the highest variety of the genus *Mortierella* species was established on clover roots. The following species were isolated: *M. humicola* Oudem., *M. candelabrum* Tiegh. et Le Monn., *M. hyalina* (Harz) W. Gams, *M. polycephala* Coem. On winter rape roots, besides the mentioned species, *M. sclerotiella and M. verticillata* Linnem were identified. After 14.5 months of crop root decomposition all fungi of the genus Moritriella disappeared, but after 19.5 months (in spring) their spread was noted again, and at the end of the study (after 26.5 months), on the roots of winter rape, the fungal species *M. sterilis* B. S. Mehrotra et B. R. Mehrotra and *M. alpina* Peyronel., not discovered in the previous trials, were established. The genus *Rhizopus* micromycetes, most often *R. oryzae* Went ex Prins. Geerl. and *R. stolonifer* (Ehrenb. ex Fr.) Vuill, were more spread in the first periods of study (after 7.5 and 14.5 months). One of the constant components of the micromycetes communities was *Mycelia sterilia*.

DISCUSSION

It has been reported that spreading of micromycetes on plant residues (roots, stems, and leaves) depends on the plant species and its chemical composition [5, 29]. In our study, the biggest number of micromycetes colony forming units was found on red clover roots; it was less on rape and the least on winter wheat roots. Micromycetes were developing better on winter than on spring rape roots. Such different micromycetes

spreading on decomposed roots of various crops were typical of all the periods of study.

Most authors indicate that micromycetes population spread depends on plant root decomposition, the carbon and nitrogen ratio of decomposed substrate and environmental conditions [30, 31]. In our study, a positive and very strong dependence of micromycetes colony forming units on the decomposition of winter rape root ($r = 0.92–0.98$, $P \leq 0.01$) was established. During the decomposition of crop root residues, the ratio of carbon and nitrogen in them decreases. It outlines the course of decomposition, i. e. mineralization [10]. During 7.5 months of winter rape root decomposition the C : N ratio in them decreased by 43.6%. First of all organic compounds of a high energetic potential and easily accessible for microorganisms were decomposed. In the residues containing much carbon, due to nitrogen deficiency carbon is more intensively decomposed, so the energetic activity of microorganisms weakens [32]. When the carbon of decomposed residues is in the form accessible to microorganisms, in aerobic conditions their activity and nitrogen immobilization are stimulated [33]. In this way the mobile nitrogen is saved. However, the amount of nitrogen accessible for microorganisms in the soil temporarily decreases. In our study, microorganisms rapidly used up the small amount of nitrogen accumulated in winter wheat roots and started decomposing carbon, therefore, the spread of micromycetes changed. Thus, the smallest number of micromycetes colony forming units was established on winter wheat roots at the end of the second cold root decomposition period in the soil (after 19.5 months). The dependence of the number of micromycetes colony forming units on the carbon and nitrogen ratio was very strong negative: $r = –0.97$, $P \leq 0.01$, and on winter rape roots strong negative ($r = – 0.83$, $P \leq 0.01$).

The largest number of micromycetes colony forming units was established after 26.5 months of decomposition, at the end of the warm period, on clover roots when their decomposition was 84.4% and C : N ratio 7.5. The increased micromycetes spreading in this period on winter wheat and winter rape roots can be explained by both C : N ratio reduction and lignin degradation and by a better accessibility to the cell cytoplasm. It has been indicated that soil fungi use as a carbon source lignin decomposition substances [34]. During the warm periods, the dependence of micromycetes colony forming units on winter rape root decomposition was very strong positive ($r = 0.98$, $P \leq 0.01$) and on the carbon nitrogen ratio very strongly negative ($r = –0.99$, $P \leq 0.01$). The correlation between the C : N ratio in winter wheat roots and the number of micromycetes colony forming units was weak negative ($r = –0.48$, $P \leq 0.01$).

After 26.5 months of decomposition, a great micromycetes variety was established. In every period of our study, the composition of micromycetes community genera and especially species changed. A high *Penicillium* genus species variety (13–16 species) was established at the beginning of investigation and after 7.5 months of crop root residue decomposition. In the warm season, *Penicillium* used up the nutrients suitable for them, and in the autumn a large part of them disappeared. On the crop roots remained immunely resistant species: on clover – *Penicillium chrysogenum,* on winter wheat – *P. variabile,* on rape – *P. capsulatum*. Some new species of the genus *Penicillium* appeared only on certain decomposed crop root substrates: clover – *P. verruculosum* Peyronel., wheat – *P. commune* Thom, rape – *P. godlewskii* K. M. Zalessky, *P. simplicissimum* (Oudem.) Thom.

Micromycetes of the genus *Fusarium* parasitize on most plants but are especially harmful for cereals. Their harm depends on the immune properties of the host plant, phytopathogen population density and microorganism community composition. The spread certain species of the genus *Fusarium* spreading is limited or their viability is reduced by secondary metabolites evoluted from separate *Trichoderma* species [35]. The decomposition of organic substances is slowed down by resins, tannins, terpenes, glucosides contained in the composition of plant residues [1]. Glucosides are typical of rape. In the decomposed rape residues, the enzyme myrosinase becomes more active, and under its influence glucosides dissociate to isotocyanates, thiocyanates, vinylthiooxazolidone which are known for their toxic properties [36]. For this reason, on the decomposing rape roots, in all periods of our study, a lower *Fusarium* variety (3–4 species) was established than on clover and wheat roots (6–10 species).

Fungi of *T. harzianum* and *T. hamatum* species, widely spread in Lithuanian soils [37], were found on all the study crop residues. *T. viride* species fungi were isolated from wheat and rape roots only during the first periods of crop residue decomposition. The further disappearance of fungi of these species from the micromycetes communities partially can be explained by sensitiveness to temperature (hot weather in the summer of 2006). A specific character of *Trichoderma* fungi spread depending on the nature of crop roots has been established. *T. longibrachiatum* fungi were found only on winter and spring rape, *T. aggressivum f. europaeum* – on winter rape, *T. spirale* – on spring rape, winter wheat and clover, *T. crassum* – on winter wheat and winter rape, *T. brevicompactum* Kraus et al. – on winter wheat roots. The mentioned genus *Trichoderma* species have been identified in Lithuania for the first time. At the end of investigation, after 26.5 months of crop root residues decomposition, the *Penicillium* species variety was reduced 4.5 times; cellulose and lignin decomposers from *Fusarium, Trichoderma, Verticillium, Mucor, Oidiodendron, Paecilomyces, Acremonium, Mortierella* genera remained active. Thus, abundant development of *Trichoderma* micromycetes on decomposed rape roots suppresses conditionally phytopathogenic *Fusarium, Rhizoctonia, Verticillium, Alternaria* fungi spreading and at the same time improve the phytosanitary condition of soil.

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AUGALŲ ŠAKNŲ LIEKANAS DIRVOŽEMYJE SKAIDANČIŲ MIKROMICETŲ BENDRIJŲ STRUKTŪRA IR RŪŠINĖS SUDĖTIES KINTAMUMAS

S a n t r a u k a

Augalų liekanų skaidymosi tyrimai buvo atlikti 2004–2006 m. LŽŪU Bandymų stoties lauko bandymuose, giliau drenuotame karbonatingame sekliai glėjiškame rudžemyje (RDg8-k2) – [*Endocalcari-Epihypogleyic Cambisol* (*sicco*)] (CMg-p-w-can). Tirtos skirtingos trukmės laikotarpiais (7,5; 14,5; 19,5; 26,5 mėn.) dirvoje skaidomos augalų šaknų liekanos: 1) žieminių rapsų (*Brassica napus* L. ssp. *oleifera biennis* Metzg.), 2) vasarinių rapsų (*Brassica napus* L. ssp. *oleifera annua* Metzg.), 3) žieminių kviečių (*Triticum aestivum* L.), 4) raudonųjų dobilų (*Trifolium pratense* L.). Tyrimų tikslas – nustatyti mikromicetų bendrijų ant dirvožemyje skaidomų žieminių ir vasarinių rapsų šaknų liekanų struktūrą ir rūšinės sudėties kintamumą lyginant su žieminių kviečių ir dobilų šaknimis.

Nustatyta, kad mikromicetų paplitimas ant skaidomų augalų šaknų labiausiai priklausė nuo augalo rūšies. Daugiausiai mikromicetų pradų aptikta ant raudonųjų dobilų šaknų, mažiau – ant rapsų šaknų ir mažiausiai – ant žieminių kviečių šaknų. Nustatyta mikromicetų pradų skaičiaus priklausomybė nuo šaknų susiskaidymo (pagal koreliacinę-regresinę analizę tiesinis stiprus ir labai stiprus ryšys) bei nuo skaidomo substrato C : N (atvirkštinis stiprus ir labai stiprus ryšys). Nustatytas *Trichoderma* genties grybų rūšių specifinis paplitimas, priklausantis nuo augalų šaknų prigimties. Pirmą kartą Lietuvoje identifikuotos šios genties rūšys: *T. longibrachiatum* – ant žieminių ir vasarinių rapsų, *T. aggressivum f. europaeum* – ant žieminių rapsų, *T. spirale* – ant

vasarinių rapsų, žieminių kviečių ir dobilų, *T. crassum* – ant žieminių kviečių ir žieminių rapsų, *T. brevicompactum* – ant žieminių kviečių šaknų. Ant skaidomų rapsų šaknų vyravo antibiotikus gaminantys *Trichoderma* genties mikromicetai (*T. harzianum, T. aggressivum f. europaeum, T. viride, T. hamatum* ir kt.), sąlyginai apribojantys fitopatogeninių *Fusarium, Rhizoctonia, Penicillium, Alternaria* ir kitų grybų vystymąsi. Visais tyrimo laikotarpiais ant rapsų šaknų buvo nustatyta mažiau *Fusarium* genties rūšių nei ant dobilų ir kviečių. Galima teigti, kad gausus *Trichoderma* grybų paplitimas ant rapsų liekanų, rapsų šaknų cheminė sudėtis bei jų skilimo produktų ypatumai yra svarbūs veiksniai, lemiantys fitosanitarinę dirvožemio būklę.

Raktažodžiai: augalų šaknų liekanos, skaidymas, mikromicetai, anglies ir azoto santykis