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Dedicated to Professor Alina Skirgiełło on the occasion of her ninety-fifth birthday

Studies on ectomycorrhizal basidiomycete in pine forest on the Lithuania–Poland transboundary region

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The diversity of ectomycorrhizal fungi and sporocarps abundance were investigated in 2003-2005 at nine permanent study plots in a 50-year-old pine forest. Diversity of ectomycorrhizal fungi consist of 53 taxa and the majority of them belonged to the genera *Cortinarius, Russula, Amanita* and *Tricholoma*. The most frequent species, whose fruit bodies were found in each study plot, were *C. cibarius, L. necator L. rufus, P. involutus, R. aeruginea, T. saponaceum* and the most abundant species which made the main part of total sporocarp yield were *C. cibarius* and *P. involutus*. The lowest species richness of ectomycorrhizal fungi was in study plots with the densest cover of grasses. Maximum of species over the fruiting period was characteristic for October and for September. It was noticed that some species virtually never occurred together at the same plot (e.g. *C. cibarius* and *H. aurantiaca*), meanwhile others occurred together quite frequently (e.g. *H. aurantiaca* and *X. badius*).

Key words: ectomycorrhizal fungi, species richness, sporocarps abundance, pine forest

INTRODUCTION

Wild mushrooms are becoming more important as a non-timber forest product and there is a need for more site-specific data on the fungi ecology and factors that influence species diversity and production of sporocarp. Macrofungi especially ectomycorrhizal ones are organisms vitally important to the forest ecosystem. Ectomycorrhizae (EM) plays a key role in nutrient cycling and energy flow of temperate and boreal forests (Smith, Read 1997). The best known mycobionts of EM belong to the Basidiomycota. Host specificity plays an important role for the distribution of ectomycorrhizal fungi. Under natural condition wide range of ectomycorrhizal fungi develop ectomycorrhizal symbiosis with *Pinus sylvestris*. *P. sylvestris* is one of the main components of coniferous forests in Lithuania. Conifer make 58.8 % of Lithuanian woodland territory, 36.4 % of the territory is occupied by *P. sylvestris* and 22.4 % by *Picea abies* (Navasaitis et al. 2003). Variations in fungal species richness, distri-

bution, and sporocarp abundance among different forest sites have been observed and may be attributed to microclimatic and macroclimatic factors, soil properties, vegetation parameters etc. Forest age has been observed to be an important factor determining the composition of ectomycorrhizal fungi (Dighton, Mason 1985; Molina et al. 1992; Ohenoja 1993; Dahlberg et al.1997). We investigated assemblage structure of ectomycorrhizal fungi associated with 50–years–old *P. sylvestris.* The objectives of present study were: 1) to perform inventory of ectomycorrhizal fungus diversity, 2) to examine sporocarp abundance of ectomycorrhizal fungi aiming to determine dominant species in the investigation territory, 3) to obtain a quantitative estimate of the relative contributions of dominant ectomycorrhizal species to assemblage structure in 50-years-old *P. sylvestris* forest situated in Lithuanian–Poland transboundary region.

MATERIALS AND METHODS

Study site. The study was carried out in permanent study site, situated in Lazdijai district, southern Lithuania (125 – 135 m a.s.l.) (Fig. 1). The mean air temperature was 6.4° C and mean annual precipitation – 550 mm. This territory is located in Lithuanian – Poland transboundary region were access for people is prohibited. This factor is important for obtaining objective investigation data because most of the ectomycorrhizal fungi are edible and intensively collected. Nine study plots (1 – 9) were set in the 50 year-old pine forest of the *Cladonio-Pinetum sylvestris* Juraszek 1927 association. Area of each study plot was 900 m^2 (30x30 m). Dominant tree species was *Pinus sylvestris* L. In some locations *Betula pendula* Roth., *Quercus robur* L. were intermixed. The shrub layer was predominantly by *Juniperus communis* L., with

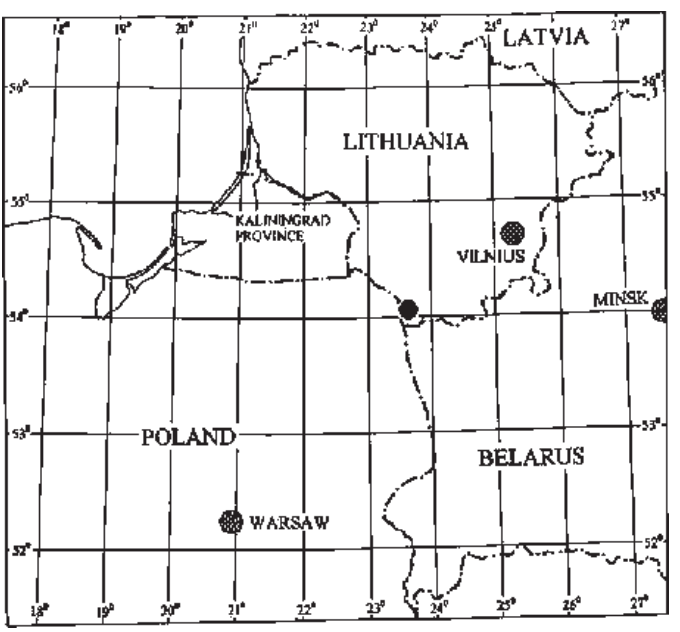

Fig. 1. Location of study area- ●.

Table 1
Vegetation cover (%) of study plots (evaluation according to Braun-Blanquet method)

Vegetation groups	Plots								
	1	2	3	4	5	6	7	8	9
Trees	50	60	60	60	50	50	60	70	60
Shrubs	60	50	10	10	10	10	15	15	20
Herbaceous plants	50	30	10	10	10	10	20	20	40
Mosses, lichens	80	70	80	70	70	70	70	80	80

occasional *Q. robur*, *Frangula alnus* Mill., *Sorbus aucuparia* L. About 80 % of study area was occupied by mosses and lichens (Tab. 1). Dominant species of mosses were *Pleurozium schreberi* (Brid.) Mitt., *Dicranum polysetum* Sw., lichens – *Cladonia rangiferina* (L.) Weber ex F. H. Wigg., *Cladonia arbuscula* (Wallr.) Flot.

Qualitative and quantitative analyses of ectomycorrhizal fungi. Species composition of fungi was inventoried at each selected forest plot every second or third week during the vegetation period in 2003 – 2005. Investigation started at the beginning of June and lasted until the first snowfall. Identification of specimens was carried out according to Moser (1983), Skirgiełło (1991, 1998), Hansen and Knudsen (1992), Urbonas (1997, 2001, 2005) using a microscope "Jenaval Carlzeiss Jena". Voucher specimens of infrequent species found within this study are deposited in the fungal collections of the herbarium of the Institute of Botany (BILAS). Number of fruit bodies collected in each investigation plot was counted. Sporocarps were weighted and biomass of fresh sporocarps (kg/ha) was calculated.

Soil analyses. Soil samples for chemical analyses were taken with soil corer of 4.5 cm diameter and 6 cm depth in August of each investigation year. The representative sample for each research plot was prepared of 18-20 randomly taken sub-samples from each plot. These soil samples were dried and sieved before the following analyses. The concentration of nitrogen and phosphorus was determined photometrically using the photometer "SPEKOL11", of potassium – applying flame photometer "FLAPHO41", and the content of humus was ascertained colorimetrically (Mineev 1989). Soil pH_{KCL} was measured potentiometrically with a glass electrode in a 1.0 M KCl suspension.

Meteorological data were obtained from the State Meteorological Service.

Data analysis was carried out using the software PC-ORD4 (McCune, Mefford 1999).

RESULTS AND DISCUSSION

Chemical characteristics of soil. Chemical composition of soil directly influences functioning of fungi in the ecosystem. Therefore the concentration of main nutrient elements – N, P, K, humus and soil pH was determined. Analysing obtained data some differences were observed between study plots. The highest concentration of N, K and humus was determined in the 9. investigation plot (Tab. 2). These concentrations were several times higher comparing to the other study plots. 9. plot is situated in the lowermost position of study area and perhaps this resulted in such a soil composition. The 8. plot distinguished by the lowest concentration of P. Higher

Table 2
Chemical composition of soil (dw) from the study plots

Plots	N (%)	P (%)	P_2O_5 (mg/kg)	K (mg/kg)	Humus (%)	pH (KCL)
1	0,073	0,027	116,8	38,3	3,75	3,82
2	0,039	0,035	150,2	36,1	3,79	3,76
3	0,035	0,027	85,8	27,7	2,94	3,78
4	0,029	0,034	118,4	21,0	2,71	3,73
5	0,079	0,026	100,8	31,2	2,89	3,66
6	0,075	0,029	107,1	26,1	2,91	3,69
7	0,033	0,037	132,7	18,9	2,05	3,82
8	0,058	0,014	49,4	29,3	2,97	3,43
9	0,197	0,028	104,6	81,0	8,02	3,47

concentration of this element was determined in the 2. and 7. plots. This is especially important for ectomycorrhizal fungi because concentration of nutrients alters ectomycorrhizal formation and community structure (Avis et al. 2003; Stankevičienė 2003; Tarvainen et al. 2003; Edwards et al. 2004; Harrington, Mitchell 2005). Other values were more or les similar comparing them between study plots.

Diversity of ectomycorrhizal fungi and sporocarp abundance. The diversity of ectomycorrhizal fungi recorded in 2003-2005 at nine permanent study plots consisted of 53 taxa (Tab. 3). Most species belonged to the genus *Cortinarius* – 12 species (23%) and *Russula* –10 (18%). Five species were found of *Amanita* and *Tricholoma* genera, four *Lactarius* and three *Hebeloma* of. Other genera were represented by 1-2 species. *Cantharellus cibarius, Lactarius necator, L. rufus*, *Paxillus involutus, Russula aeruginea* and *Tricholoma saponaceum* were found in each study plot. Taylor (2002) investigating diversity of ectomycorrhizal fungi in central Sweden in a 50–year-old *Pinus sylvestris* stand found very similar species richness - 56 species.

Species of ectomycorrhizal basidiomycetes from the investigated pine forest could be distributed into several groups according to the abundance of formed fruit bodies. 22 species formed only small amount of fruit bodies – 10 sporocarps/investigation period. Rarest species, of which only 1 – 2 fruit bodies/investigation time were found, were *B. pinophilus, C. causticus, C. bovinus, C. delibutus, C. evernius, H. pusila, L. bicolor, L. vietus, R. claroflava, R. decolorans* and *T. felleus*. Seven species were the most abundant and formed more than 100 fruit bodies in study plots/investigation time. It was *C. cibarius, P. involutus, Rozites caperata, L. rufus, Hygrophoropsis aurantiaca, Cortinarius mucosus* and *R. aeruginea*. Those were dominant species in 50-year-old pine forest. Intermediate group was composed of 24 species which formed 11-100 fruit bodies in study area per investigation period. It is known that each forest type has its own dominant mushroom species and that dominants make main biomass of sporocarps and usually determine harvest in different forest types (Skryabina, Sennikova 1981). *C. cibarius* was the most abundant species, its sporocarps made about a half of the total numbers of all collected sporocarps of ectomycorrhizal fungi. *P. involutus* harvested quite abundantly also. The number of fruit bodies formed by this species made about a quarter and their biomass – about a half of the total amount of fruit bodies. Studies on fungal fruit bodies in mixed

Table 3
Diversity of ectomycorrhizal fungi species and sporocarp abundance in study forest

Species	Species code	Sum [1]	Mean [2]	Maximum [3]	S [4]
Amanita citrina (Schaeff.) Pers.	Amcitr	51	5.7	23	4
A. fulva (Schaeff.) Fr.	Amfulv	26	2.9	25	2
A. muscaria (L.) Lam.	Ammusc	53	5.9	35	7
A. porphyria Fr.	Amporf	28	3.1	12	5
A. rubescens Pers.	Amrube	42	4.7	15	7
Boletus edulis Bull.	Boledu	18	2	10	5
B. pinophilus Pilát et Dermek	Bolpin	2	0.2	1	2
Cantharellus cibarius Fr.	Cantc	5526	614	1095	9
Cortinarius alboviolaceus (Pers.) Fr.	Cortal	4	0.4	2	2
C. armillatus (Alb. et Schwein.) Fr.	Cortam	11	1.2	7	2
C. causticus Fr.	Cortca	1	0.1	1	1
C. bolaris (Pers.)Fr.	Cortbo	3	0.3	3	1
C. bovinus Fr.	Cortbv	1	0.1	1	1
C. cinnamomeus (L.) Fr.	Cortci	5	0.6	3	2
C. delibutus Fr.	Cortde	2	0.2	2	1
C. evernius (Fr.) Fr.	Cortev	2	0.2	2	1
C. mucosus (Bull.) Cooke	Cortmu	146	16	45	8
C. salor Fr.	Cortso	3	0.3	3	1
C. semisanguineus (Fr.) Gillet	Cortse	5	0.6	4	2
C. traganus (Fr.) Fr.	Corttr	44	4.9	18	6
Hebeloma crustuliniforme (Bull.) Quél.	Hebecr	44	4.9	44	1
H. longicaudum (Pers.) P. Kumm.	Hebelo	3	0.3	3	1
H. pussilum J. E. Lange	Hebepu	1	0.1	1	1
Hygrophoropsis aurantiaca (Wulfen) Maire	Hygaur	173	19	65	7
Laccaria bicolor (Maire) P. D. Orton	Lacbic	2	0.2	2	1
Lactarius necator (Bull.) Pers.	Lactne	96	10.7	30	9
L. rufus (Scop.) Fr.	Lactru	392	43.6	104	9
L. torminosus (Schaeff.) Gray	Lactto	28	3.1	20	2
L. vietus (Fr.)	Lactvi	1	0.1	1	1
Leccinum scabrum (Bull.) Gray	Leccsc	19	2.1	7	8
Paxillus involutus (Batsch.) Fr.	Paxinv	2475	275	552	9
Rozites caperata (Pers.) P. Karst.	Rozcap	405	45	117	7
Russula adusta (Pers.) Fr.	Rusadu	24	2.7	20	3
R. aeruginea Fr.	Rusaer	99	11	29	9
R. claroflava Grove	Ruscla	1	0.1	1	1
R. decolorans (Fr.) Fr.	Rusdec	2	0.2	1	2
R. emetica (Schaeff.) Pers.	Ruseme	79	8.8	24	7
R. nigricans (Bull.) Fr.	Rusnig	3	0.3	2	2
R. rhodopoda Zvára	Rusrod	14	1.6	9	5
R. sanguinea (Bull.) Fr.	Russan	3	0.3	3	1
R. vesca Fr.	Rusves	32	3.6	13	8
R. xerampelina (Schäff. ex Secr.) Fr.	Rusxer	4	0.4	4	1
Sarcodon imbricatus (L.) P. Karst.	Sarimb	92	10.2	42	5
Suillus bovines (Pers.) Kuntze	Suilbo	27	3	14	6
S. variegatus (Sw.) Kuntze	Suilva	24	2.7	11	5
Tylopilus felleus (Bull.) P. Karst.	Tylofe	1	0.1	1	1
Tricholoma equestre (L.) P. Kumm.	Triequ	90	10	27	5
T. pesundatum (Fr.) Quél.	Tripes	4	0.4	3	2
T. portentosum (Fr.) Quél	Tripo	40	4.4	14	7
T. saponaceum (Fr.) P. Kumm.	Trisap	78	8.7	32	9
T. sejunctum (Sowerby) Quél.	Trisej	9	1	4	3
Xerocomus badius (Fr.) Kühner	Xerbad	85	9.4	40	8
X. subtomentosus (Fr.) Fr.	Xersub	34	3.8	19	7

Explanations: [1] – sum of sporocarps collected in all study plots/whole investigation period (2003–2005); [2] – mean of sporocarp number yielded in one plot; [3] – maximum of sporocarps yielded in one plot; [4] – number of plots in which the species yielded.

forest in Switzerland showed that abundance of ectomycorrhizal sporocarps varied over the years of the investigation and ranged from 58 to 5559 per vegetation season (Straatsma et al. 2001). Present studies showed that sporocarp abundance of ectomycorrhizal fungi in pine forest in Lithuania over the study period varied from 2487 to 5025 sporocarps per year. A total of 10808 fruit bodies of ectomycorrhizal basidiomycetes during the three year study period were collected. It made 894 kg (fresh weight)/8100m^2 or 1104 kg/ha per investigation time or 368 kg/ha per one vegetation season.

According to the sporocarp abundance study plots were distributed into three groups. About 1,500 fruit bodies per investigation period were collected in the richest plots (4, 5, 8). Plots 1 – 3, 6 and 9 took intermediate position and yielded 1000 – 1270 fruit bodies. The least amount, a little over 300 sporocarps was found in the plot 7. It was 3-5 times lower than in the other study plots. As it was mentioned above, *C. cibarius* was the dominant species in the investigated pine forest. However, amount of fruit bodies of chanterelle in the plot 7 was very low (only 9 fruit bodies/ investigation period) and this determined the minimum total yield of sporocarps in this plot. Meanwhile *H. aurantiaca* and *Xerocomus badius* fruited in this plot most abundantly. It is interesting that the reliable positive correlation (r = 0.7) between the abundance of sporocarps of the last-mentioned species and reliable negative correlation between *C. cibarius* and *H. aurantiaca* (r = -0.70) also *C. cibarius* and *X. badius* (r = -0.76) was determined. Analysing distribution of species (these which yielded more than 5 fruit bodies per investigation period) and their frequency in study plots several groups were selected (Fig. 2). It means that is likely to find species which belong to the same group (e.g. *C. cibarius, R. vesca*, also *A.porphyria, S. bovinus* etc.)

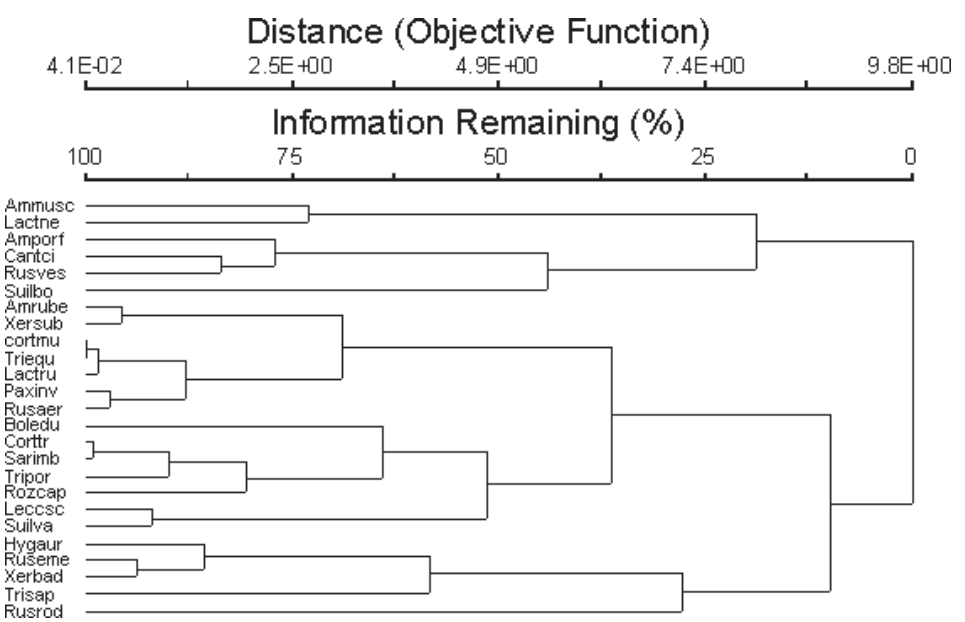

Fig. 2. Groups of ectomycorrhizal fungi species (yielded more than 5 fruit bodies per investigation time) which frequently occurred together at the same plot.

in the same area with the similar relative abundance and on the contrary the species from different groups prefer different growing conditions. These observations were made after only three year field study. Aiming to confirm this relationship the investigation time possibly should be prolonged and at present it can be stated more as a tendency than the strong correlation. Agerer et al. (2002) while investigating correlations between ectomycorrhyzae formed by different ectomycorrhizal fungi species noted that some species show no common occurrence within shot distance, however, other indicate rather high values of common occurrence. The reasons of this phenomenon are not quite clear. The chemical composition of soil is an important factor, because varying demands of different species for soil conditions are expressed. It was determined that changes of different soil ions concentrations (N, P, S, K, Na, pH etc.) influence above- and belowground community structure of ectomycorrhizal fungi (Tyler 1985; Agerer 1990; Erland, Söderström 1990; Fransson et al. 2000; Lilleskov et al. 2002; Agerer, Gotlein 2003, Tarvainen et al. 2003; Stankevičienė, Urbonas 2006). In the present studies the plot with the minimum amount of fruit bodies was distinguished by the lowest concentration of K, humus and the highest value of pH (Tab. 2).

The lowest species richness (15 species) was determined in the plot 1 which characterized by the highest coverage of herbaceous plants (grasses) (50 %) and shrubs (60 %) (Tab. 1). On the other hand, the plots with the maximum species (4. plot – 32 species; 9. – 30; 3. – 29) were characterized by the lower coverage of shrubs (10 %) and grasses (10 %, except plot 9, where coverage of grasses was 40 % and shrubs – 20 %). Fungal species composition seems to be strongly determined by soil chemical properties, vegetation type, structure and age of the forest stand especially in the case of ectomycorrhizal species.

Phenology. Fruit bodies were monitored every second or third week between June and October. The start of fruiting varied strongly between the species. The longest period of fruiting was characteristic to *C. cibarius* and *P. involutus.* Fruit bodies of these species started to growth in June and fruited till the end of the vegetation season. Long fruiting period was characteristic also for *L. scabrum, B. edulis*, also for some species from genus *Amanita*, *Lactarius*, *Russula, Xerocomus.* Fruit bodies of mentioned fungi started to growth in July or August. Species from genus *Cortinarius, Tricholoma, Hebeloma* also *Sarcodon imbricatus* were found from September and their fruiting period was relatively short. Maximum species richness was characteristic for September in 2004 and for October in 2003, 2005. The dynamics curve of richness and abundance per vegetation season (June-October) was sinusoid (Fig. 3 A, B). It means that minimum value of species or sporocarps in one period (eg. a month) was compensated by the maximum in the next period and, on the contrary, the maximum was replaced by the minimum values in the following fruiting period. The dynamic of the species richness and fruit bodies abundance in 2003 and 2004 demonstrated similarities of these parameters in different fruiting periods. However, comparing appointed values between the three year period, it was noted that the curve of 2005 quite differed from 2003, 2004. The reason of these differences probably was meteorological conditions, because the curves of the dynamic of species richness, sporocarp abundance and precipitation demonstrated similar patterns in the period of 2003 – 2005 (Fig. 3 A, B, C). It is known that the highest

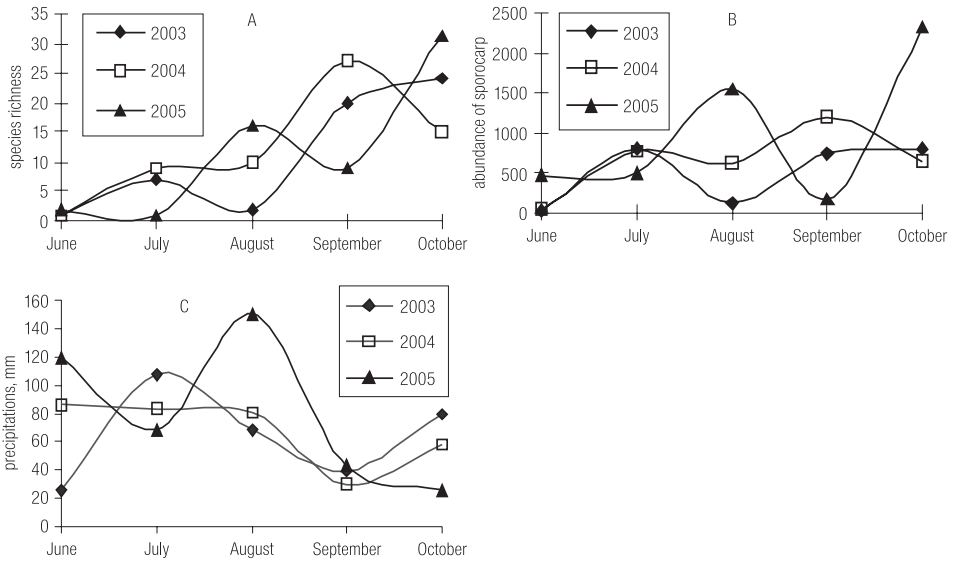

Fig. 3. Dynamics of species richness (A), sporocarp abundance (B) and precipitation (C) during vegetation period of 2003–2005.

abundance of sporocarp mainly depends on the climatic conditions (Senikova, 1984) and it is highly probable to that abundant fruiting period will supervene when the short drought period is followed by abundant precipitation (Kasparavičius, Stankevičienė 2004).

Summarizing the results of investigation it can be said that diversity of ectomycorrhizal fungi in 50-year-old pine forest consisted of 53 taxa and the majority of them belong to the genera *Cortinarius, Russula, Amanita* and *Tricholoma*. The most frequent species, which fruit bodies were found in each study plot were: *C. cibarius, L. necator L. rufus, P. involutus, R. aeruginea, T. saponaceum* and the most abundant species which made the main part of total yield of sporocarps in study forest were *C. cibarius* and *P. involutus*. The lowest species richness of ectomycorrhizal fungi showed study plots with the highest coverage of herbaceous plant and shrubs. Maximum of species over the fruiting period was characteristic for October and also for September. Sporocarp abundance was also influenced by meteorological conditions. It was noticed that some species virtually never occurred together at the same plot (e.g. *C. cibarius* and *H. aurantiaca*), meanwhile others occurred together quite frequently (e.g. *H. aurantiaca* and *X. badius*).

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