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FUNGI ASSEMBLAGES OF THE PHYLLOSPHERE OF EASTERN PURPLE CONEFLOWER (*Echinacea purpurea* (L.) Moench.) FERTILIZED WITH AMMONIUM SULPHATE

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Abstract. The mycobiota composition of plant phyllosphere depends on atmospheric factors as well as on the physiological properties of plant. In the recent years the infections of the eastern purple coneflower by *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Fusarium oxysporum*, *Verticillium dahliae* and by *Erysiphe cichoracearum* have been observed. The development of these pathogens is dependent on the species composition of the phyllosphere organisms, but it is also affected by the form and the level of nitrogen nutrition. The undertaken study aimed at the analysis of the mycobiota composition of the coneflower phyllosphere as dependent on the rate of ammonium sulphate used in the plant fertilization. The experiment was set up in 2007–2009 at Psary near Wrocław. Two fertilizer rates were applied: 50 kg N·ha^{-1} and 200 kg N·ha^{-1}. The fungal taxa most abundantly isolated from the leaf surface of coneflower are: *Cladosporium* spp., *Alternaria alternata*, yeast-like colonies, *Fusarium* spp. and *Epicoccum nigrum*. These fungi make up > 90% of the isolated colonies., with *C. herbarum* and *A. alternata* as the dominant components of the assemblages. The dominant species within *Fusarium* spp. is *F. equiseti*. The species composition of the assemblages of fungi in the coneflower phyllosphere is variable and dependent on the course of the weather. The fertilization rate of ammonium sulphate does not affect the species composition of the phyllosphere fungi assemblages of the coneflower.

Key words: *Cladosporium* spp., *Fusarium* spp., nitrogen dose

INTRODUCTION

The eastern purple coneflower (*Echinacea purpurea* (L.) Moench.), a perennial plant of *Asteraceae* family, can be cultivated in majority of soils that occur in Poland [Bie-

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siada et al. 2006]. Kordana et al. [1998] have demonstrated that both the amount and quality of the harvested material of this plant as well as the concentration of the biologically active ingredients in it are dependent on the course of the weather during the vegetation period and on the plant fertilization. The factor that determines the yielding level is the mineral fertilization. According to Górecki [1996], in soils moderately rich in nutrients the rates of nitrogen, potassium and phosphorus should amount to 60–80 kg $N \cdot ha^{-1}$, 40–60 kg $P_2O_5 \cdot ha^{-1}$, and 80–100 kg $K_2O \cdot ha^{-1}$ respectively. *Echinacea purpurea* is a perennial plant and as such it requires annual nitrogen nutrition. Nitrogen rates of approximately 50 kg $N \cdot ha^{-1}$ usually suffice for a satisfactory yield. However, the yield increase resulting from the increased plant nutrition is not always accompanied by the adequate quality of the harvested plant material. The results of agrotechnological experiments concerning the effect of different forms and rates of nitrogen fertilizers on the plant yield are ambiguous [Berbeć et al. 1998, Kordana et al. 1998, Biesiada et al. 2006]. Some of them suggest that higher nitrogen rates may negatively affect the health status of the plants [Pląskowska and Pusz 2010].

The form in which nitrogen is supplied to the plant affects the rate of building up its photosynthetic apparatus, iron take up from the soil and enzyme activity. These in turn, with peroxidase activity in particular, affect the plants` health status [Rivero et al. 2003, Assimakopoulolu 2006]. The physiolgical processes taking place in the epidermis may determine the composition of epiphytic mycobiota. Plant phyllosphere is a natural habitat populated by microorganisms, including species that are pathogens of plants, animals and humans [Bansal et al. 1988]. The species composition of such mycobiota depends on relative air humidity and temperature, pH of the phyllosphere solution when the plant surface becomes wetted, as well as on the plant species and its phenological stage [Chruściak 1974].

Fungi of the genera *Fusarium*, *Rhizoctonia*, *Alternaria*, *Cladosporium*, *Phoma* and *Botrytis* are commonly found on many herbaceous plant species, potentially causing reduction in the quality of the harvested plant material [Machowicz-Stefaniak et al. 2002a, 2002b, Machowicz-Stefaniak et al. 2003, Machowicz-Stefaniak and Zalewska 2007, Zimowska and Machowicz-Stefaniak 2004].

There has been evidence in the recent years that the coneflower becomes infected by *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Fusarium oxysporum*, *Verticillium dahliae* and *Erysiphe cichoracearum* 1999 [Chang et al. 1997a, Chang et al. 1997b, Chang et al. 1998, Peichowski et al. 1997, Putnam and Crowe 1999, Sholberg et al. 1999]. These pathogens may affect the coneflower yield in terms of its amount and quality, but their development largely depends on the phyllosphere species composition and on the level of nitrogen nutrition of the plant. A possible disturbance of the fragile equilibrium between the epiphytic pathogens and saprotrophic microorganisms may result in plant disease.

The study aimed at analysing the species composition of the phyllospheric mycoflora of the eastern purple coneflower, as dependent on the rates of ammonium sulphate.

MATERIAL AND METHODS

The field experiment was set up in 2007–2009 in Psary near Wrocław. The square plots of 1m width were arranged in randomized sub-blocks design in 3 replicates. *Echinacea purpurea* spacing was 50 × 30 cm. The ammonium sulphate was used in two variants. In the first variant, the complete rate of 50 kg $N \cdot ha^{-1}$ was applied before the start of plant vegetation. In the second variant, 200 kg $N \cdot ha^{-1}$ was applied in two, equal, split rates: one before the start of plant vegetation and the other one – at the leaf rosette stage.

In July every year, each time after 3 days without rain, from each plot 6 leaves of the coneflower were sampled from the central part of each plant. From every leaf 4 rings of 1 cm^2 surface area were cut. The rings were shaken for 10 minutes with 10 ml of distilled water in a glass volumetric flask (amplitude 4–250 cycle). After that, 1 ml portions of the wash water were transferred to 10 sterile Petri dishes and covered with melted Martin medium cooled to 46°C. After incubation for 7–14 days at 25°C, the plates were examined microscopically. Subcultures on potato-dextrose agar (PDA) slants were made for preservation of cultures. Sporulating fungi were identified on the basis of their morphology according to the available literature [Guba 1961, Raper et al. 1965, Raper et al. 1968, Rifai 1969, Zycha and Siepmann 1969, Ellis 1971, Sutton 1980, Nelson et al. 1983].

RESULTS AND DISCUSSION

The fungi most abundantly represented among the taxa isolated from the coneflower phyllosphere in every year of the study were *Cladosporium* spp., *Alternaria alternata*, yeast-like colonies, *Fusarium* spp. and *Epicoccum nigrum*. They made up > 90% of the total number of the isolated colonies (tab. 1, fig. 1). The observations of other authors confirm the incidence of these taxa in the phyllosphere of herbaceous plants, crop plants and grass cultures [Abdel-Hafez 1984, Chmiel and Paśmionka 1998, Cwalina--Ambroziak et al. 2001, Kita 1988, Machowicz-Stefaniak et al. 2003, Machowicz-Stefaniak and Zalewska 2007, Mazur and Szczeponek 2005, Patkowska 2003, Wagner 2000, Zimowska and Machowicz-Stefaniak 2004].

The abundance of the fungi varied between the years of the study. However, the highest number of the isolated colonies was observed in 2007, in which the amount of precipitation was the highest compared to the other two seasons (tab. 1, 2, fig. 2). Kita [1988] had demonstrated similar results while investigating the phyllosphere mycobiota of sunflower in a year with extraordinary high precipitation.

In the first year of the study, 2007, *A. alternata* predominated in the sampled material, whereas in the next two seasons *Cladosporium* spp. was the most often isolated taxon (tab. 1, fig. 1). Chmiel and Paśmionka [1998], Patkowska [2003], as well as Machowicz-Stefaniak and Zalewska [2007] have found the fungi of *Cladosporium* genus abundantly infecting cultures of soy bean, orchard grass (*Dactylis glomerata* L.), annual bluegrass (*Poa annua* L.), spring barley, oilseed rape, sugar beet and potato. On the other hand, Machowicz-Stefaniak et al. [2002a] and Machowicz-Stefaniak and

Zalewska [2007] have demonstrated that the leaves of the lemon balm (*Melissa officinalis* L.) and dill (*Anethum graveolens* L.) are commonly infested by *A. alternata*.

The fungus most frequently isolated in 2007 was *C. cladosporioides*, but in 2008–2009 *C. herbarum* was recovered from the plant phyllosphere most often (tab. 1). Chmiel and Paśmionka [1998] have found that *C. herbarum* ocurred abundantly on grass cultures, whereas Cwalina–Ambroziak et al. [2001] collected large number of colonies of *C. herbarum* and *C. cladosporioides* from the leaves of potato.

Table 1. Frequency of fungi isolated from the phyllosphere of *Echinacea purpurea* fertilized with ammonium sulphate

Tabela 1. Liczebność grzybów wyizolowanych z fyllosfery *Echinacea purpurea* nawożonej saletrą amonową

Fungi species Gatunek grzyba	Years – Lata						Total Razem
	2007		2008		2009		
	ammonium sulphate rate dawki saletry amonowej (kg N·ha^{-1})						
	50	200	50	200	50	200	
Alternaria alternata (Fr.) Keissl.	169	174	70	56	12	14	495
Aspergillus flavus Link		1					1
Aspergillus niger Tiegh.				1	5	5	11
*Botrytis cinerea (*Pers.) ex. Fr.	4	6			7	2	19
Cladosporium cladosporioides (Fresen.)	78	110	41	36	7	7	279
Cladosporium herbarum (Pers.) Link	1	4	146	151	171	187	660
Epicoccum nigrum Link	29	44	21	9	26	8	137
Fusarium avenaceum (Fr.) Sacc.			1	1			2
Fusarium culmorum (W. G. Smith) Sacc.	11	4	1	1			17
Fusarium equiseti (Corda) Sacc.	15	153	41	5	11	3	228
Fusarium graminearum Schwabe	7	7					14
Fusarium oxysporum Schlecht.	1	1	1	1			4
Fusarium sporotrichioides Sherb.	10	5					15
Mucor hiemalis Wehmer	2			2	5	1	10
Penicillium notatum Westling	2	1					3
Penicillium urticae Bainier	1			3	1		5
Pestalotia epilobii Rolland & Fantrey	2	2					4
Phoma chrysanthemicola Hollós			9	1			10
Phoma medicaginis Malb. & Roum			9	15			24
Rhizopus nigricans Ehrenb.	4	4	4	1		7	20
Sclerotinia sclerotiorum (Lib.) de Bary	3	2			6	5	16
Trichoderma hamatum (Bonord.) Brainier		6					6
Trichoderma harzianum Rifai	2	1		4			7
Trichoderma viride Pers.			2		116		118
Ulocladium botrytis Preuss		4	8		54		66
Non-sporulating colonies	9	19	85	7	15	13	148
Yeast fungi	82	32	17	69	10	45	255
Total – Razem	432	580	456	363	446	297	2574

In 2007 *Cladosporium* spp. were apparently isolated more often from the plants treated with the higher rate of nitrogen (200 kg N·ha^{-1}) and the colonies of *A. alternata* were obtained in the highest number in 2008, from the plants fertilized with 50 kg N·ha^{-1} (tab. 1, fig. 1). Based on the paper by Cwalina-Ambroziak et al. [2001] one may conclude that *A. alternata* infests most abundantly the phyllosphere of potato when medium level of nitrogen fertilization is applied (60 kg N·ha^{-1}), whereas the same nitrogen rate can limit the development of *Cladosporium* spp.

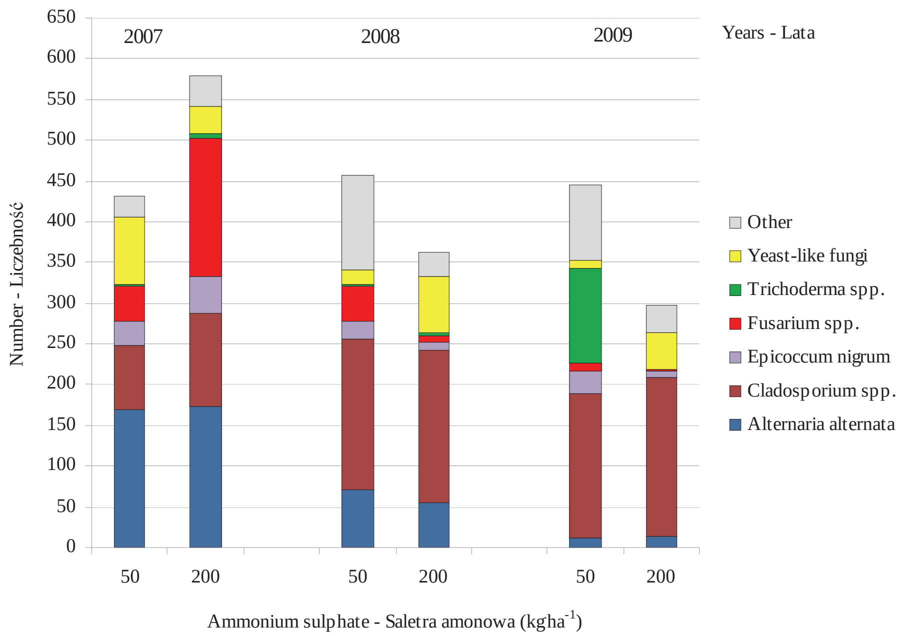

Fig. 1 Fungi most frequently isolated from the phyllosphere of Echinacea purpurea fertilized with ammonium sulphate

Rys. 1 Grzyby najliczniej wyizolowane z fyllosfery Echinacea purpurea nawożonej saletrą amonową

Relatively high numbers of yeast-like fungi were isolated from the leaf surface of the investigated plants. Majority of the isolates were collected in 2007 from the plants nourished by the lower rate of nitrogen. In the other years the yeast-like colonies infested more abundantly the phyllosphere of coneflower plants fertilized with 200 kg N·ha^{-1} (tab. 1, fig. 1). Yeast-like colonies are commonly found on the leaf surface of many plant species [Abdel-Hafez 1984, Cwalina-Ambroziak et al. 2001, Cwalina-Ambroziak et al. 2007, Kita 1988]. As demonstrated by Chmiel and Paśmionka [1998], more such colonies can be isolated from older leaves than from the young ones. In our experiment the leaves to be analysed by mycologist were sampled from the middle portion of the plants, shortly before the flower harvesting. It seems likely that the observed degree of the leaves infestation by the yeast-like organisms is typical for the phyllosphere of the eastern purple coneflower.

The fungi of *Fusarium* genus had infested the leaf surface of the coneflower most intensively during the first year of the study and their species composition was the most diverse of all the isolated taxa (tab. 1, fig. 1). The rainfall, that in 2007 was the highest in the entire period of the study, as well as the temperature, that had exceeded long-term average, created fafourable conditions for the development of *Fusarium* spp. (tab. 2). Mazur and Szczeponek [2005] obtained similar results while isolating *Fusarium* spp. abundantly from the above-ground parts of garden angelica, *Angelica archangelica* L. (*Archangelica officinalis* Hoffm.).

Table 2. Temperature and rainfall in the study years
Tabela 2. Temperatura i opady w latach badań

Lata – Years			Miesiące – Months						
			I	II	III	IV	V	VI	VII
Temperatura Temperature (°C)	średnia miesiąca monthly mean temperatures	2007	4.9	-1.9	6.8	11.8	15.9	19.7	20.3
		2008	3.7	4.2	5.1	10.2	16	20.7	21.7
		2009	2.5	0.5	4.9	13.8	15.8	17.0	21.0
	średnia za lata 1970–2000 long-term mean in 1970–2000		-1,0	0.1	3.7	8.1	13.9	16.7	18.5
Opady Rainfall (mm)	suma miesiąca monthly sum of rainfall	2007	29.9	53.1	25.1		53.4	101.1	108.3
		2008	56.1	2.9	30.21	71.2	1.4	23.5	85.7
		2009	12.2	21.0	22.8	4.2	54.2	79.3	115.9
	średnia za lata 1970–2000 long-term mean sums of rainfall in 1970–2000		30.5	24.6	33.2	31.9	49.9	64.9	75.4

Fusarium spp. isolated from the plant leaf surface had been represented by six species: *F. equiseti*, *F. culmorum*, *F. graminearum*, *F. sporotrichioides*, *F. avenaceum* and *F. oxysporum*. *F. equiseti* predominated in the coneflower phyllosphere in every year of the study (tab. 1). Machowicz-Stefaniak and Zalewska [2007], as well as Zimowska and Machowicz-Stefaniak [2004] have recorded abundant infestations of the above-ground parts of dill (*Anethum graveolens* L.) and St John's wort (*Hypericum perforatum* L.) by *F. equiseti*. According to Machowicz-Stefaniak et al. [2002a and 2002b], *F. equiseti, F. culmorum* and *F. avenaceum* can be dangerous pathogens of a number of herbal crops, as they cause wilting and dying of seedlings. Other species of *Fusarium* spp., namely *F. culmorum*, *F. oxysporum*, *F. graminearum*, *F. avenaceum* and *F. sporotrichioides*, were isolated from the coneflower phyllosphere sporadically (tab. 1). On the other hand, Mazur and Szczeponek [2005] have demonstrated that these fungi species may be abundantly isolated from the above-ground parts of garden angelica. Perhaps then, the proportion of different *Fusarium* species found in phyllosphere is dependent on plant species.

In the first year of the study *Fusarium* spp. infested to the greatest extent the phyllosphere of plants fertilized with the higher nitrogen rate. During the other years these fungi were isolated more often from the plants treated with ammonium sulphate at the rate of 50 kg $N \cdot ha^{-1}$ (tab. 1, fig. 1).

The fungi of *Trichoderma* genus were represented by three species: *T. viride, T. harzianum* and *T. hamatum*. The predominant species of *T. viride* was isolated at greatest numbers from the leaves of coneflower nourished by the lower nitrogen rate, in 2009. In 2007–2008 all *Trichoderma* species were isolated sporadically, irrespectively of the fertilization rate variant (tab. 1, fig. 1). Kita [1988] had been isolating *T. hamatum* and *T. koningii* from the phyllosphere of sunflower and Abdel-Hafez [1984] isolated *T. viride* from the phyllosphere of the fern *Ceterach officinarum*.

During the three-year study a relation has been observed between the abundance of fungi of *Fusarium* spp. on the one hand and those belonging to *Trichoderma* genus – on the other. In 2007–2008, when *Fusarium* spp. species were isolated very abundantly, the *Trichoderma* fungi had been found only occasionally in the coneflower phyllosphere. The humid and warm 2007 favoured the development of *Fusarium* spp. The decrease in the isolate number of *Fusarium* spp. in 2009 can be related to the drought, prevailing until the beginning of April that year, as well as to the abundant occurence of *Trichoderma* spp. in the same phyllosphere samples (tab. 1, 2, fig. 1). *Trichoderma* species are commonly considered as antagonists of the pathogenic fungi such as *Fusarium* spp. [Galletti et al. 2002].

Another fungal taxon, the one fairly often isolated in 2008, was *Phoma* spp. (tab. 1). Machowicz-Stefaniak et al. [2003] as well as Machowicz-Stefaniak and Zalewska [2007] have recorded fungi of this genus on the above-ground parts of dill (*Anethum graveolens* L.) and caraway (*Carum carvi* L.).

Other fungi species identified in the course of the study, as *Pestalotia epilobii*, *Aspergillus* spp., *Penicillium* spp., *Mucor* spp., *Rhizopus* spp., *Botrytis cinerea* and *Sclerotinia sclerotiorum* were isolated sporadically (tab. 1).

CONCLUSIONS

1. The dominant fungal taxa in the phyllosphere of the eastern purple coneflower, *Echinacea purpurea* (L.) Moench., were *Cladosporium herbarum* and *Alaternaria alternata*.

2. The coneflower phyllosphere favours the development of *Fusarium* spp.

3. The species predominant within the *Fusarium* genus was *F. equiseti*.

4. The species composition of the assemblages of fungi in the coneflower phyllosphere is variable and dependent on the course of the weather.

5. The fertilization rate of ammonium sulphate does not affect the species composition of the phyllosphere fungi assemblages of the coneflower.

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ZBIOROWISKA GRZYBÓW FYLLOSFERY JEŻÓWKI PURPUROWEJ (*Echinacea purpurea* (L.) Moench.) NAWOŻONEJ SALETRĄ AMONOWĄ

Streszczenie. Skład mikobiota zasiedlających powierzchnię roślin jest uzależniony od czynników atmosferycznych oraz właściwości fizjologicznych rośliny. W ostatnich latach stwierdzono porażenie jeżówki purpurowej przez *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Fusarium oxysporum*, *Verticillium dahliae* i *Erysiphe cichoracearum*. Rozwój tych grzybów zależy od składu mikroorganizmów fyllosfery oraz od formy i dawki nawożenia azotem. Celem podjętych badań była analiza składu mikobiota fyllosfery jeżówki w zależności od dawek nawożenia saletrą amonową. Doświadczenie założono w latach

2007–2009 w Psarach pod Wrocławiem. Zastosowano dwie dawki nawożenia: 50 N kg·ha^{-1} i 200 N kg·ha^{-1}. Z powierzchni liści jeżówki purpurowej, najliczniej wyizolowano: *Cladosporium* spp., *Alternaria alternata*, kolonie drożdżoidalne, *Fusarium* spp. i *Epicoccum nigrum*. Grzyby te stanowiły ponad 90% wyizolowanych kolonii. Dominantami były *C. herbarum* i *A. alternata*. W obrębie rodzaju *Fusarium* dominującym gatunkiem był *F. equiseti*. Skład zbiorowisk grzybów fyllosfery jeżówki był zmienny i zależał od warunków pogody. Dawka nawożenia saletrą amonową nie miała wpływu na skład zbiorowisk grzybów.

Słowa kluczowe: *Cladosporium* spp., *Fusarium* spp., dawka azotu

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