ACTA Acta Sci. Pol., Hortorum Cultus 12(4) 2013, 153-162

FUNGI ASSEMBLAGES OF THE PHYLLOSPHERE OF EASTERN PURPLE CONEFLOWER (*Echinacea purpurea* (L.) Moench.) DEPENDING ON THE RATE OF NITROGEN

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Abstract. The fungi of phyllosphere may affect the plants health status and the yield obtained from the crop. Species composition of mycobiota infesting the plant`s phyllosphere is not only dependent on the atmospheric factors, but also on the nitrogen fertilization. The field experiment was set up in Psary near Wrocław, in the years 2007–2009. The aim of the study was to determine the species composition of the phyllospheric fungi of the purple coneflower depending on the nitrogen rate: 50 kg N·ha^{-1} and 200 kg N·ha^{-1}. The fungi species most abundantly isolated from the coneflower phyllosphere were *Cladosporium herbarum*, *Alternaria alternata* and *Cladosporium cladosporioides*. The abundance of the isolated fungi taxa was significantly lower in 2008, compared to the other two years. The atmospheric conditions in 2007 encouraged development of *Fusarium* spp. During the three-year experiment *Gibberella intricans* predominated in the coneflower phyllosphere. The lower level of nitrogen (50 kg N·ha^{-1}) favoured the increased incidence of *Cladosporium* spp., *Gibberella intricans*, *F. sporotrichioides, F. oxysporum, F. culmorum, Gibberella avenacea* and *Gibberella zeae*.

Key words: Cladosporium spp., Alternaria alternata, Fusarium spp., nitrogen fertilization

INTRODUCTION

The eastern purple coneflower (*Echinacea purpurea* L.) has been extensively used in herbalism and cosmetology. The green parts of the plant as well as its root and rhizomes are exploited as crude herbal material for the preparation of medicines. They should therefore be characterised by the excellent quality in terms of the active ingredi-

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ents content and the plants` health status. However, in spite of the bacteriastatic and fungistatic properties of certain herbal plants, disease symptoms may be seen on their organs, being brought about by bacteria, fungi and viruses [Zimowska and Machowicz-Stefaniak 2004]. During the growth of the coneflower crops the pathological alterations have been observed on plants, that are caused by *Sclerotinia sclerotiorum*, *Botrytis cinerea*, *Fusarium oxysporum*, *Verticillium dahliae* and *Erysiphe cichoracearum* [Chang et al. 1997a,b, Chang et al. 1998, Peichowski et al. 1997, Putnam and Crowe 1999, Sholberg et al. 1999].

The phyllosphere-infesting fungi may affect the plants health status and, consequently, impact on the amount and quality of the yield obtained from the crop. At the prevalent atmospheric conditions that are favourable for the pathogens, the resultant disturbance in the equilibrium between epiphytic pathogens and saprotrophic microorganisms can precipitate plant disease. However, the quantitative, as well as species composition of the mycobiota infesting the plant`s phyllosphere is not only dependent on the atmospheric factors, but also on the physiological traits of the plant and on its fertilization [Bansal et al. 1988, Chruściak1974].

As the atmospheric and edaphic conditions prevalent in Poland are adequate ones to the cultivation of the eastern purple coneflower, the yield quantity and quality are principally shaped by the cultural factors and plant nutrition [Biesiada et al. 2006, Węglarz and Karaczun 1996]. For the coneflower crops are retained in the same field for 3 to 4 years, therefore the soil tillage preceding their planting should be done diligently [Hetman et al. 1996]. Kordana et al. [1998] have demonstrated that the coneflower responds more readily to the deficiency or excess of nitrogen, than it does with respect to the phosphorus or potassium. The nitrogen deficiency reduces the yield of plant material, whereas supplying the nutrient in excess – debilitates the crop`s flowering. The lush, bushy plants are weak and limp and their leaves become easily affected by atmospheric factors and by fungi and herbivorous insects [Hetman et al. 1996]. According to many authors [Moszczyńska and Pląskowska 2005, Pląskowska 2005, Moszczyńska et al. 2007] high nitrogen fertilization promote infection of plants by pathogens. The fertilization rates appropriate for the soil of a moderate nutrient content should amount to 60–80 of N, 40–60 of P_20_5 and 80–100 of K_2O [kg·ha^{-1} pure ingredient]. Berbeć et al. [1998], Biesiada et al. [2006] and Kordana et al. [1998] consider 100 kg N·ha^{-1} an optimal level of plant nutrition with nitrogen.

The aim of the present study is to determine the species composition of the phyllospheric mycobiota of the eastern purple coneflower depending on the application of nitrogen rate.

MATERIAL AND METHODS

The field experiment was carried out in the years 2007–2009, in the Experimental Station of Vegetable Crops and Ornamental Plants of the University of Environmental and Life Sciences, located in Psary near Wrocław (51.19°N, 17.03°E). The experiment was established in one factorial design in three replications and plot area 1.0 m^2 on fine clay soil contained 1.8% of humus. Two levels of nitrogen were used: (1) 50 kg N·ha^{-1} in the form of urea 46% N – applied every year at the end of April, before the plant vegetation, and (2) 200 kg N·ha^{-1} (100 kg N·ha^{-1} before the plant vegetation had started in the form of urea – plus 100 kg N·ha^{-1} of the ammonium nitrate 34% N – as a top dressing at rosette growth stage). The coneflower was grown from transplants produced in 2006 and planted on plots in spacing of 50 × 30 cm. Tests of phyllosphere were carried out on one, two and three years old plants. The atmospheric conditions during the experiment were described in the previous publication [Moszczyńska et al. 2011]. Each year in July, after 3-day period without precipitation, 6 leaves were sampled from the central fragment of each plot. 4 discs of 1cm^2 size were cut from each leaf. The discs were stirred for 10 min in 50 ml round-bottom flasks with 10 ml of distilled water. Following, 1 ml volumes of the rinse water were poured onto sterile Petri dishes and covered with Martin agar medium at 46°C [Kita 1988]. After two weeks of incubation at room temperature, the developed fungal colonies were inoculated onto PDA medium slants and identified taxonomically, following the monographic keys of Guba [1961], Raper et al. [1965], Raper et al. [1968], Rifai [1969], Zycha and Siepmann [1969], Ellis [1971], Sutton [1980] and Nelson et al. [1983]. In order to analyse data statistically, the log-linear model was employed that allows for verification of the null hypothesis. The model also makes it possible to assess, after rejection of the insignificant interactions, the effect of particular factors on the variation of the investigated population [Goodman 1971]. The partial association between the experimental factors such as years, fertilization and fungi abundance encompases the remaining interactions as well. The fungi species selected for the log-linear analysis are *Cladosporium* ssp., *Alternaria alternata*, the yeast-like fungi, *Fusarium* spp., *Epicoccum nigrum*, *Phoma* spp. and *Trichoderma* spp.

RESULTS AND DISCUSSION

The fungi most abundantly isolated from the phyllosphere of *Echinacea purpurea* in the course of the 3-year study are *Cladosporium* spp., *Alternaria alternata,* the yeast-like fungi, *Fusarium* spp. and *Epicoccum nigrum* (tab. 1, fig. 1). Their incidence was dependent on atmospheric conditions observed in the years 2007–2009; the species appeared at significantly lower numbers in 2008 (tab. 2). Similarly, Kita [1988] and Cwalina-Ambroziak and Wróbel [2004], working on the sunflower and potato phyllosphere, isolated fungal colonies in lower numbers during the warm and dry seasons.

The phyllosphere fungi in our study were isolated most abundantly from the coneflower grown on plots fertilized with nitrogen at the rate of 50 kg N·ha^{-1}, whereas the leaves sampled from the plots fertilized at the rate of 200 kg N·ha^{-1} "yielded" a lower number of the fungal colonies (tab. 1, fig. 1). Furthermore, significant interaction was found between the study year on the one hand and the fertilization rate and the total abundance of the isolated fungi. At the higher N rate the incidence of the leaf-infesting fungi was lower (tab. 2). Similar results were reported by Cwalina-Ambroziak and Wróbel [2004], from the study of potato phyllosphere. The lower fungal incidence in the phyllosphere of plants fertilized with N at the higher rates may be related to polyphenol content in plant tissue. Biesiada et al. [2006] have found a higher level of polyphenol concentration in the leaves of the coneflower fertilized with 100 kg N·ha^{-1}, compared to those in which the rate of 50 kg N·ha^{-1} had been used.

Table 1. The abundance of fungi species isolated from the phyllosphere of *Echinacea purpurea* depending on the rate of nitrogen

Fungus species	Years						Total
	2007		2008		2009		
	rate of nitrogen (kg N·ha^{-1})						
	50	200	50	200	50	200	
Alternaria alternata (Fr.) Keissl.	167	182	39	40	66	38	532
Aspergillus brasiliensis Varga, Frisvad	1	24			2	3	30
Botrytis cinerea Pers.				2	3	4	9
Cladosporium cladosporioides (Fresen.)	94	93	69	47	80	53	436
Cladosporium herbarum (Pers.) Link	3		83	80	301	76	543
Epicoccum nigrum Link	28	37	18	11	17	12	123
Gibberella avenacea R.J. Cook			1	1	2		4
Fusarium culmorum (W.G. Sm.) Sacc.	8	11	1	4			24
Gibberella intricans Wollenw.	36	19	12	20	5	4	96
Gibberella zeae (Schwein.) Petch		2					2
Fusarium oxysporum Schltdl.	22	6	2	8			38
Fusarium sporotrichioides Sherb.	33	5					38
Mucor hiemalis Wehmer		2					2
Penicillium chrysogenum Thom				10	2	3	15
Penicillium thomii Maire		1					1
Penicillium griseofulvum Dierckx					1		1
Pestalotia epilobii Rolland & Fautrey		2					2
*Phoma chrysanthemicola (*Hollós)				4		27	31
Phoma medicaginis Malb. & Roum			3	16			19
Rhizopus stolonifer (Ehrenb.) Vuill.	4	2	10	2	7	6	31
Sclerotinia sclerotiorum (Lib.) de Bary	8					2	10
Trichoderma hamatum (Bonord.) Brainier.	3	1					4
Trichoderma harzianum Rifai		1					1
Trichoderma viride Pers.			1	10		6	17
Ulocladium botrytis Preuss	1	10		5			16
Non-sporulating colonies	9	40	45	10	14	61	179
Yeast-like fungi	12	60	77	42	70	22	283
Total	429	498	361	312	570	317	2487

During the first year of the study, the fungus found in greatest numbers of isolates from the leaf surface was *A. alternata*, whereas in the next two years – *Cladosporium* spp. (tab. 1, fig. 1). Other authors investigating the phyllosphere of vascular plants had also listed the fungi species belonging to these two genera as the taxa most abundantly represented in their studies [Kita 1988, Machowicz-Stefaniak et al. 2002a,b, Cwalina--Ambroziak et al. 2007]. In our work *Cladosporium* species predominated in the purple coneflower phyllosphere; their percent proportion in the isolated fungal material amounted to 20%, 41% and 58% in 2007, 2008 and 2009 respectively (fig. 1). The research of Abdel-Hafez [1984] and of Sharma [2011] confirm that *Cladosporium* spp.

occur profusely on plant leaves. The significant interaction between the fertilization level of the coneflower plants and the number of isolated fungi species, observed in our study, indicates their variable response to the applied nitrogen rates. The lower used dose of urea (50 kg N·ha^{-1}) favoured the enhanced incidence of *Cladosporium* spp., which has been confirmed by the log-linear analysis (tab. 2). The same may also be concluded from the work of Cwalina-Ambroziak and Wróbel [2004] on potato phyllosphere, who had found that at the lower level of urea fertilization these fungal taxa were isolated more frequently. With respect to the species assortment, our isolates in 2007 contained mostly *C. cladosporioides*, whereas in 2008 and 2009 the species most abundantly recognized was *C. herbarum* (tab. 1). Abdel-Hafez [1984] reports an extensive infestation of the leaves of ferns by *C. herbarum*. On the other hand, Kita [1988] and Cwalina-Ambroziak et al. [2001] most often isolated *C. cladosporioides* from the leaves of sunflower and potato.

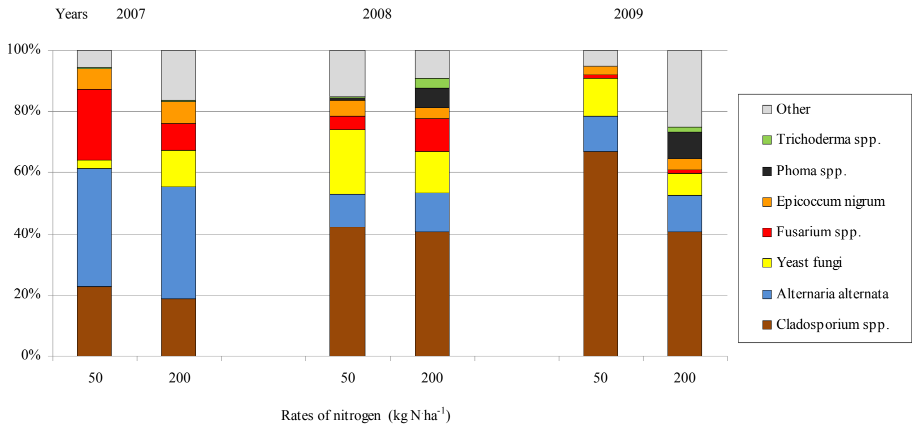

Fig. 1. Proportion of fungi most abundantly isolated from the phyllosphere of *Echinacea purpurea* depending on the rate of nitrogen

The next species, with respect to the number of isolates, has been *A. alternata*. Its percent proportion in the sampled material has reached 38% in 2007. Nevertheless, in 2008–2009 the species made up only ca. 12% of all the isolated colonies (fig. 1). It has been concluded, from the work of Machowicz-Stefaniak et al. [2002b] and Cwalina-Ambroziak et al. [2007], that *A. alternata* predominates in the phyllosphere of thyme (*Thymus* L.) and in that of potato. Mazur and Szczeponek [2005] confirm, that the infestation of wild celery (*Angelica archangelica* L., syn. *Archangelica officinalis* Hoffm.) plants by this fungus is a commonplace. Yet, other authors have found the infestation by this species infrequent on the leaves of potato and ferns [Abdel-Hafez 1984, Cwalina-Ambroziak et al. 2001]. The presence of *A. alternata* on its host plant may result in spots or blotches on the plants green parts, which deteriorates the quality of plant material, or may lead to the leaves dessication and their loosing by the plant [Machowicz-

-Stefaniak 2001]. It seems likely that the abundance of *A. alternata* is primarily related to the host plant age and to the prevalent atmospheric conditions in a particular growth season. Moreover, although it is clear from the study of Cwalina-Ambroziak et al. [2001], that the lower urea fertilization favours development of the pathogen, in the course of our three-year work no statistically significant relation was found between the nitrogen rate applied to the crop and the frequency of this fungus in the isolated mycological material.

The yeast-like fungi, *Fusarium* spp. and *Epicoccum nigrum* had been isolated in lower numbers in the years 2007–2009; their percentage proportion to all the sampled material had reached 11%, 8% and 5% respectively (fig. 1). Abdel-Hafez [1984] reported a sparse incidence of the yeast-like fungi and sporadical isolation of *E. nigrum*, from the leaves of ferns. Nevertheless, the yeast-like fungi may prevail in the phyllosphere of plants such as potato [Cwalina-Ambroziak et al. 2001, Cwalina-Ambroziak et al. 2007]. Some authors consider these fungi as pioneer organisms, that stimulate the subsequent development of *Alternaria* spp. [Cwalina-Ambroziak et al. 2001]. No such sequential pattern has been observed in our study. In 2007, at the higher dose of nitrogen *E. nigrum* and the yeast-like fungi were isolated most numerous. Conversely, in the next two years more of them had been isolated from the plants nourished by 50 kg N·ha^{-1} (tab. 1, fig. 1). However, the different variants of nitrogen fertilization did not affect the incidence of *E. nigrum* significantly. Likewise, according to Cwalina-Ambroziak et al. [2001], variable nitrogen rates do not always produce treatment variation with respect to the abundance of yeast-like fungi.

Table 2. Log-linear analysis of the variation in the abundance of *Cladosporium* spp., *A. alternata*, yeast-like fungi, *Fusarium* spp., *E. nigrum*, *Phoma* spp. and *Trichoderma* spp., depending on the year of study and on the nitrogen fertilization rate

Experimental factors	Degrees of freedom	Chi^2 Partial association	Significance level (p)	Chi^2 Marginal association	Significance level (p)
Years (1)	2	43.30**	0.00001	43.34**	0.00001
Fertilization (2)	1	45.01**	0.00001	45.01**	0.00001
Fungi species (3)	6	2007.97**	0.00001	2007.97**	0.00001
Interaction (1) × (2)	2	70.63**	0.00001	75.91**	0.00001
Interaction (1) × (3)	12	552.09**	0.00001	557.36**	0.00001
Interaction (2) × (3)	6	95.54**	0.00001	100.81**	0.00001

** associations and interactions characterized by $p < 0.01$ are statistically significant

Fusarium species made up 15% of the overall number of colonies in 2007 (fig. 1). At the same time, they had been at their maximum abundance level during the whole three-year study period. *Fusarium* development in 2007 had apparently taken advantage from the high summer rainfall and from the temperatures considerably exceeding those of the long-term average [Moszczyńska et al. 2011]. On the other hand, in 2008–2009 these fungi species had been obtained sporadically and their percent proportion in the entire material never exceeded 7% in those years (fig. 1). The data collected by Abdel-Hafez [1984], Cwalina-Ambroziak and Wróbel [2004], as well as by Cwalina--Ambroziak et al. [2007] confirm the infrequent incidence of *Fusarium* spp. on the leaves of ferns and potato. In our study, *Fusarium* spp. has been represented by six species and these are *Gibberella intricans* (syn. *F. equiseti*)*, F. culmorum, Gibberella zeae* (syn. *F. graminearum*)*, F. sporotrichioides, Gibberella avenacea* (syn. *F. avenaceum*) and *F. oxysporum*. The species composition of *Fusarium* spp. was the most diverse in 2007–2008, whereas in 2009 only *G. intricans* and *G. avenacea* were isolated. During the three-year experiment *G. intricans* predominated in the coneflower phyllosphere (tab. 1). Contrastingly, Cwalina-Ambroziak and Wróbel [2004] had found *F. culmorum, G. avenacea* and *F. concolor* most abundantly isolated from the phyllosphere of potato, with *G. intricans* being found there only sporadically. *G. intricans* has been isolated in Poland from different plant organs of St John's wort (*Hypericum perforatum* L.), coriander (*Coriandrum sativum* L.), common fumitory (*Fumaria officinalis* L.), herb hyssop (*Hyssopus officinalis* L.) and of marjoram (*Origanum majorana* L.) [Filoda et al.1998]. In Israel, *G. intricans* has been claimed the cause of extensive dying out of seedlings and mature plants of caraway (*Carum carvi*) [Reuveni 1982]. As Nelson et al. [1983] have it, forms of *Fusarium* spp. characterized by variable pathogenicity potential can be found among the essentially saprotrophic species of the genus, existant in soils. *F. culmorum* had been found in Poland on mature plants of coriander, whereas *G. avenacea* was detected infesting stem base of St John's wort and herb hyssop [Filoda et al. 1998]. In 2007 and 2009 *Fusarium* spp. had been isolated in the greatest numbers from the plants on plots fertilized with urea at the rate of 50 kg $N \cdot ha^{-1}$, and the difference between the treatments was statistically significant (tab. 1, fig. 1). Similarly, Cwalina-Ambroziak and Wróbel [2004] had isolated the highest number of *Fusarium* colonies from the phyllosphere of potato nourished by the low doses of urea.

For the reason of dosage and nutrient form of the applied nitrogen fertilizers, the taxa belonging to *Trichoderma* and *Phoma* genera were only sporadically isolated from the phyllosphere of the purple coneflower in the whole three-year study period. They were represented by the 3 species: *T. viride, T. harzianum* and *T. hamatum,* with *T. viride* as the predominating component (tab. 1, fig. 1). Also Abdel-Hafez [1984] reports an infrequent incidence, on the leaves of ferns, of *Trichoderma* spp. and their most abundant representative: *T. viride*, along with *Phoma* spp.

The remaining fungi species: *Pestalotia epilobii, Botrytis cinerea* and *Sclerotinia sclerotiorum*, as well as *Aspergillus* spp.*, Penicillium* spp.*, Mucor* spp.*,* and *Rhizopus* spp., as it is shown in table 1., had been isolated only sporadically.

CONCLUSIONS

1. The dominant fungi species of the phyllosphere of *Echinacea purpurea* are *Cladosporium herbarum*, *Alternaria alternata* and *Cladosporium cladosporioides*.

2. The atmospheric conditions prevailing in 2007 favoured the development of the *Fusarium* spp., with the predominant species of *Gibberella intricans*.

3. The lower level of nitrogen fertilization encourage increased abundance of *Cladosporium* spp. and *Fusarium* spp.

4. The variable nitrogen fertilization levels do not significantly affect the abundance of *Alternaria alternata*, *Epicoccum nigrum*, *Trichoderma* spp., *Phoma* spp., or the yeast-like fungi.

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ZBIOROWISKA GRZYBÓW FYLLOSFERY JEŻÓWKI PURPUROWEJ (*Echinacea purpurea* (L.) Moench.) W ZALEŻNOŚCI OD DAWKI AZOTU

Streszczenie. Grzyby fyllolsfery mogą wpływać na stan zdrowia roślin oraz plon uzyskiwany z uprawy. Skład gatunkowy grzybów infekujących z fyllosfery może być zależny od czynników atmosferycznych lub nawożenia azotem. Doświadczenie zostało założone w latach 2007–2009 w Psarach pod Wrocławiem. Celem badań było określenie składu gatunkowego grzybów fyllosfery *Echinacea purpurea* w zależności od zastosowanej dawki azotu 50 kg $N \cdot ha^{-1}$ i 200 kg $N \cdot ha^{-1}$. Z powierzchni liści jeżówki purpurowej najliczniej były izolowane *Cladosporium herbarum*, *Alternaria alternata* i *Cladosporium cladosporioides*. W 2008 r. uzyskano istotnie mniejszą liczebność wyizolowanych grzybów. Warunki atmosferyczne w 2007 r. były korzystne dla rozwoju grzybów rodzaju *Fusarium.* W czasie trzyletnich badań w fyllosferze jeżówki dominował *Gibberella intricans*. Mniejsza dawka azotu (50 kg $N \cdot ha^{-1}$) sprzyjała wzrostowi liczebności *Cladosporium* spp., *Gibberella intricans, F. sporotrichioides, F. oxysporum, F. culmorum, Gibberella avenacea* i *Gibberella zeae*.

Słowa kluczowe: *Cladosporium* spp., *Alternaria alternata*, *Fusarium* spp., nawożenie azotowe

Accepted for print: 8.04.2013