

## Prevalence peculiarities of airborne *Alternaria* genus spores in different areas of Lithuania

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The annual, seasonal and hourly distribution of *Alternaria* Ness. spores in the air was measured in three urban areas in Lithuania – Klaipėda, Šiauliai, and Vilnius in 2005–2006. Hirst type 7-day recording spore traps were used for spore fixation. Fungal spores were identified and counted according to 12 transverse traverse method. Duration of spore season was determined using 90 % method. Duration of season ranged from 62 days in Vilnius in 2005 to 97 days in Klaipėda in 2006. The biggest amount of *Alternaria* spores was established in summer and the least one – in winter and spring. Only solitary spores were observed in February, March and April in all aerobiological stations. The total annual amount of *Alternaria* reached 3 090 spores in Vilnius aerobiological station in 2005 and 9 718 spores in Klaipėda in 2006. The peak of sporification season was recorded in August in all locations and it was from 121 to 707 spores m<sup>-3</sup>. In this month there were observed 51.4–70.3 % of all annual spores counted. The hourly pattern of *Alternaria* spores concentration in August indicated maximum value between 13:00 and 1:00 hours. Minimal amounts were recorded at 5:00–9:00 hours.

**Key words:** *Alternaria*, fungal spores, season duration, spore concentration.

**Introduction.** *Alternaria* is an extremely common and widespread saprophyte or plant pathogenic genus, which causes big economic loses throughout the world (Corden et al., 2003; Munuera et al., 2001). The analysis of the prevalence of this fungus spores is substantial, because *Alternaria* colonises all the stages of plant growth (Mitakakis et al., 2001). It's necessary to emphasize that some species are plant pathogens, which collectively cause a range of diseases with impact on a large variety of important agronomic host plants including cereals, ornamentals, oilcrops, vegetables and fruits (Thomma, 2003). Common *Alternaria* micromycetes *A. brassicicola*, *A. brassicae*, *A. raphani*, *A. alternata* cause diseases of oilseed and turnip rapes (Petraitienė, 2005), *A. radicina*, *A. dauci* – of carrots (Survilienė, Valiuškaitė, 2006), *A. brassicicola* and *A. brassicae* – of cabbages (Survilienė et al., 2004), besides, *A. alternata*, *A. radicina* and *A. tenussisima* cause human diseases also (Lugauskas et al., 2002).

Most aeromycology investigations are conducted in towns (Corden et al., 2003; Kasprzyk et al., 2004; Stępańska, Wołek, 2005; Kasprzyk, Worek, 2006); however, some

researchers pay attention to data originating from countryside (Mitakakis et al., 2001; Brazauskiene, Petraitiene, 2006; Kasprzyk, Worek, 2006). In rural environments the spread of airborne fungal spores there was investigated to determine occurrence during the crop harvest (Mitakakis et al., 2001) or to estimate the quantity of plant pathogenic fungal spores in the air (Brazauskiene, Petraitiene, 2006). During these studies fungal spores were sampled with Burkard volumetric spore traps, which were located in the fields of crops (Brazauskiene, Petraitiene, 2006). Passive sedimentation plates are the alternative method, which was used to evaluate the concentration of fungal spores in the air. This method is more practical since allows to estimate viable micromycetes spores and to identify species. Passive sedimentation plate method is being used for the short time (Lugauskas et al., 2003), while the volumetric method allows evaluating the season of fungal spores (Stepalska et al., 1999; Corden, Millington, 2001; Corden et al., 2003; Konopińska, 2004; Oliveira et al., 2005; Kasprzyk, Worek, 2006). In Lithuania passive method is the most common. The most common micromycetes species in Vilnius during various seasons of 1996–1999 was *Alternaria alternata* (Lugauskas et al., 2003).

The aim of this work is to study the annual, seasonal and hourly distribution of *Alternaria* airborne spores in the air of three cities in Lithuania. This comparative study of coastal (Klaipėda) and landlocked (Vilnius and Šiauliai) areas of Lithuania is especially interesting, since it allows evaluating the peculiarities of spore dispersion.

**Objects, methods and conditions.** Spores were sampled at three sites, in different places, which partly represented Lithuania – in Klaipėda (Western Lithuania, coastal place), Šiauliai (Northern Lithuania, landlocked place), and Vilnius (Southern Lithuania, landlocked place). Monitoring was carried out using three volumetric Hirst-type 7-day recording spore traps with flow rate of 10 L min<sup>-1</sup>. Spore traps were located in Klaipėda – 55°45'20 N and 21°07'32 E, in Šiauliai – 55°55'36 N and 23°18' E, in Vilnius – 54°40'40 N and 25°16'05 E.

The measurements of airborne fungal spore concentration in the air were carried out by volumetric method in 2005–2006 (Lacey, West, 2006). Spore monitoring took place in Šiauliai from 4 of February to 31 of December in 2005 and from 22 of March to 11 of October in 2006. In second site, Klaipėda it took place from 28 of January to 26 of September in 2005 and from 8 of March to 12 of October in 2006. In Vilnius, it took place from 3 of February to 22 of September in 2005 and from 9 of March to 30 of September in 2006. Melinex tape, greased with a thin layer of silicone solution was changed once in a week at the same time (Mandrioli et al., 1998). The exposed tape was cut in 48 mm segments, thus each represented 24 hours period. Average daily concentrations (spores m<sup>-3</sup>) were obtained by counting spores for every two hours by twelve transverse traverse method (Sterling et al., 1999). Sample representing each day were analysed for spores of *Alternaria* by light microscope Nikon Eclipse 50i at a magnification of 400x (0.50 mm microscopic field). For microscopic fungal spore identification Lacey, West (2006) and Käärik et al. (1983) publications were used as reference books.

Estimated spore values were transformed into the number of spores in each cubic metre of air sampled per day. The duration of spore season was calculated by 90 %

method – the start of *Alternaria* spore season was defined as the date when 5 % of the seasonal cumulative spore amount was trapped, and the end of the season – as the date when 95 % of the seasonal cumulative spore amount was reached (Stepalska, Wołek, 2005; Kasprzyk, Worek, 2006). The days when *Alternaria* spore amount reached 100 spores in cubic meter of air were recorded as the peak days. The hourly distribution of *Alternaria* spores concentration was determined in August, the peak of spore season. Statistical correlation and regression analysis was carried out using Stat-Eng programme. Significant difference was assessed at a critical value of  $p < 0.05$  and  $p < 0.01$ .

**Results.** Seasonal data of *Alternaria* spores. The duration of spore season, calculated by 90 % method is shown in Table 1.

**Table 1.** Seasonal data of *Alternaria* spores in Lithuania in 2005–2006  
**1 lentelė.** *Alternaria* sporų sezono trukmė Lietuvoje 2005–2006 m.

Year Metai	Start date Pradinė data (5 %)	End date Galinė data (5 %)	Duration (days) Trukmė (dienomis)
Klaipėda			
2005	12 July Liepos 12	14 September Rugsėjo 14	65
2006	19 June Birželio 19	23 September Rugsėjo 23	97
Šiauliai			
2005	20 July Liepos 20	6 October Spalio 6	78
2006	11 July Liepos 11	18 September Rugsėjo 18	68
Vilnius			
2005	12 July Liepos 12	11 September Rugsėjo 11	62
2006	21 June Birželio 21	18 September Rugsėjo 18	90

The *Alternaria* spore season duration range was from 62 days in Vilnius in 2005 to 97 days in Klaipėda in 2006. In both years of research the most stable duration of spore season was in Šiauliai, it ranged to 68–78 days. In both years of observation spore season appeared to begin at the earliest in Klaipėda and Vilnius, whereas in Šiauliai it was late for 8 days in 2005 and 20–22 in 2006 (depending on stations). The end of *Alternaria* spore season during monitoring period in all aerobiological stations occurred in September, except Šiauliai in 2005, where spore season occurred in October.

Annual and monthly totals of *Alternaria* spores. The cumulative annual and monthly totals of daily average concentrations of *Alternaria* spores are shown in Table 2. The highest *Alternaria* monthly concentrations were observed at the end of summer, in the middle of spore season in all three locations in both years of study period. Spore sum in August constituted 51.4–65.6 % of all spores counted in

2005, and 56.4–70.3 % of all spores counted in 2006. In June and May, irrespectively the season, *Alternaria* spores occurred in the air at low concentrations and sporadically in March, April and October in all sites. A strong 95 % significant positive correlation ( $r = 0.942$ ) was obtained between the monthly *Alternaria* spore amounts in different years in Klaipėda. Similar findings were in the rest two aerobiological stations: a strong 99 % positive correlation ( $r = 0.992$ ) in Šiauliai and ( $r = 0.962$ ) in Klaipėda.

**Table 2.** Annual and monthly totals and the highest peak day concentrations of *Alternaria* spores in Lithuania in 2005–2006, (peak values in brackets)

**2 lentelė.** *Alternaria* sporų pasiskirstymas pagal metus bei mėnesius ir piko dienos koncentracijos Lietuvoje 2005–2006 m. (piko duomenys sliausteliuose)

Year Metai	Monthly total amount (spores m <sup>-3</sup> ) / Highest concentration on peak day (spores m <sup>-3</sup> ) / Bendras kiekis per mėnesį, sporos m <sup>-3</sup> / Didžiausia piko dienos koncentracija, sporos m <sup>-3</sup>					Annual totals (spo- res m <sup>-3</sup> ) Iš viso per metus, sporos m <sup>-3</sup>
	May gegužė	June birželis	July liepa	August rugpjūtis	September rugsėjis	
Klaipėda						
2005	29 / 5 /	61 / 13 /	903 / 124 /	2 674 / 306 /	796 / 74 /	4 476
2006	124 / 11 /	348 / 67 /	1 832 / 208 /	5 888 / 707 /	1 253 / 135 /	9 718
Šiauliai						
2005	39 / 8 /	66 / 10 /	735 / 126 /	4 038 / 375 /	868 / 82 /	6 160
2006	37 / 5 /	109 / 18 /	1 001 / 139 /	4 205 / 547 /	568 / 61 /	5 984
Vilnius						
2005	26 / 4 /	77 / 13 /	1 008 / 220 /	1 588 / 121 /	386 / 65 /	3 090
2006	95 / 16 /	267 / 30 /	1 365 / 249 /	3 511 / 414 /	876 / 122 /	6 223

The highest daily concentration of *Alternaria* spores was recorded in the middle of the season (August) in Klaipėda and Šiauliai aerobiological stations in both years. The highest concentrations occurred at the beginning of spore season only in Vilnius in 2005 and in Šiauliai in 2006.

The number of peak days and maximal concentration of *Alternaria* spores in aerobiological stations are shown in Table 3. *Alternaria* spores had their monthly peaks in July and August in all aerobiological stations. Concentration range in 2005 was from 121 to 306 spores m<sup>-3</sup> air, while in 2006 concentration range was considerably higher – from 122 to 707 spores m<sup>-3</sup> air.

No peak days in September of 2005 were recorded, in the same time in 2006 – only few. Only 5 days were recorded as peak days of *Alternaria* spores in Vilnius in 2005, among those 3 days in July at the start of spore season. Nevertheless, in the second monitoring year there were recorded 14 spore day peaks in this aerobiological station. The biggest number of spore peak days was determined in Klaipėda in 2006 – even 28 days.

**Table 3.** Number of *Alternaria* spore peak days and concentration of peak day in Lithuania in 2005–2006

**3 lentelė.** *Alternaria* sporų piko dienų kiekis bei piko dienų koncentracijos Lietuvoje 2005–2006 m.

Aerobiological station Aerobiologinė stotis	July Liepa		August Rugpjūtis		September Rugsėjis	
	2005 year 2005 metai	2006 year 2006 metai	2005 year 2005 metai	2006 year 2006 metai	2005 year 2005 metai	2006 year 2006 metai
Number of days with $\geq 100$ spores in $m^{-3}$ Dienų skaičius, kai $\geq 100$ sporų $m^{-3}$						
Klaipėda	3	6	8	18	-	4
Šiauliai	1	2	13	17	-	-
Vilnius	3	3	2	10	-	1
Maximal concentration of spores on peak day in $m^{-3}$ Maksimali sporų koncentracija piko dieną, $m^{-3}$						
Klaipėda	124	208	306	707	-	135
Šiauliai	126	139	375	547	-	-
Vilnius	220	249	121	414	-	122

A strong 95 % no significant positive correlation ( $r = 0.956$ ) was obtained between the maximal concentrations of spores on peak day every month in Klaipėda in different years. The similarly findings were in other aerobiological station: a strong 99 % positive no significant correlation ( $r = 0.996$ ) in Šiauliai and medium ( $r = 0.485$ ) in Vilnius.

Hourly pattern of *Alternaria* spores. Maximum value of spore concentration in August was found in midnight, between 23:00–1:00 in Klaipėda in 2005, but next year of monitoring the maximal values were established between 17:00–19:00. Minimal spore counts were noted in 5:00–7:00 in both years of investigations.

The hourly analysis of samples corresponding air of Šiauliai in August in 2005 demonstrated the highest concentrations in the afternoon, 13:00–15:00. In the second year of monitoring maximal values were determined between 17:00–19:00. The lowest *Alternaria* spore amounts here reached at night between 3:00–7:00 in 2005 and 2006.

In Vilnius aerobiological station clear daily peak of spore concentration in August wasn't recognized, concentration of spores was equally dispersed and only obscure increase was noticed in 19:00–3:00 time interval. The least amount of spores was counted in the morning at 9:00. In the second year there were differences: maximal values appeared to be between 17:00 and 19:00, while minimal at 5:00–7:00.

Roughly, the highest concentrations of *Alternaria* spores were between 15:00 and 1:00, while minimal values were observed at 5:00–9:00 in August in Lithuania.

**Discussion.** It is possible to distinguish two regularities based on annual spore counts, comparing the results obtained from three aerobiological stations: 1) the lowest spore concentrations were in Vilnius, while higher in Šiauliai and Klaipėda aerobiological stations; 2) the interannual differences were smaller in Šiauliai than in the rest aerobiological stations – spore amount in Klaipėda in 2006 was more than twice as high as in 2005 and spore amount were 1.6 as high in 2006 as in Vilnius in 2005.

On the basis of 2005–2006 investigation, the highest total annual amount of *Alternaria* airborne spores occurred in Vilnius aerobiological station in 2006 (6223 spores) and in Klaipėda in 2006 (9718 spores). Such abundance in Šiauliai maybe was caused by availability of host plants, because *Alternaria* is a common pathogen of cereals, vegetables, ornamentals, oilcrops, vegetables and fruits (Thomma, 2003). The investigations conducted in urban and rural environments in Poland confirm the preliminary hypothesis that daily concentrations, mean concentrations and seasonal sums of the studied fungal spores are lower in city air, than in areas with lower urbanisation level (Kasprzyk, Worek, 2006). Our sites according to town size and distance from its centre to the spore traps can be arranged by urbanisation level increase as follows: Klaipėda–Šiauliai–Vilnius. Easily can be noticed, that spore sums from all the investigation period according to sites are in decreasing order: 14 194–12 144–9 313.

*Alternaria* airborne fungal spores occur in Lithuania throughout nearly the whole year. In February, March and April spores occur in air sporadically and in May, June concentrations of its conidia are usually low; the maximum falls in August, less – in July. According to Oliveira et al. (2005), the lowest concentrations of *Alternaria* spores were registered in winter and the highest concentrations were found in summer and early autumn in Porto (Portugal) in 2003. Spore concentrations were determined in summer in other places of Europe also: in Rzeszów, Lublin and other cities of Poland (Kasprzyk et al., 2004; Konopińska, 2004; Stępańska et al., 1999; Stępańska, Wołek, 2005), in Derby (England), Zagreb (Croatia) and other (Corden et al., 2003; Peternel et al., 2004). Corden et al. (2003) estimated that in two England towns Derby and Cardiff abundantly *Alternaria* spores are released in July, August and September, the main months for harvesting. Similar observations were made by Stępańska and Wołek (2005) in Cracow, Poland. The main months for release of *Alternaria* and other airborne fungal spores are July, August and September. These are the main months for combine harvesting and grass moving.

Konopińska (2004) recorded in Liublin shorter time of *Alternaria* spore maximal value between 17:00–20:00, but longer minimal amounts value time: 0:00–7:00. Peternel et al. (2004) recorded in Zagreb minimal amounts of *Alternaria* spores on early morning, in 6:00. Munuera et al. (2001) observed that distribution of spore concentrations through the day was similar in 1993–1998 in Murcia, in Southern Spain: the maximal value of spore concentration was found in evening, at about 19:00, the lowest spore concentration was noted in early morning, at about 8:00. The same authors proposed that this late afternoon maximum could be related to a late release of spores from local sources or delay in sampling of spores released earlier and being transported to the sampler from rural areas (15–30 km away).

*Alternaria* spore prevalence monitoring can be useful part of Integrated Disease Management programs.

**Conclusions.** 1. The highest concentrations of *Alternaria* airborne spores in Lithuania were measured in the middle of spore season, i. e. in August. 51.4–70.3 % of annual spore amount was observed in this month.

2. Depending on site and year, there were from 5 (Vilnius, 2005) to 28 (Klaipėda, 2006) peak days with high concentration of *Alternaria* spores in the air.

3. Hourly pattern of *Alternaria* spore concentration in August indicated the maximal value between 15:00 and 1:00, while minimal amounts were observed at 5:00–9:00.

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### **Ore plintančių *Alternaria* genties grybų sporų sklaidos ypatumai skirtingose Lietuvos vietovėse**

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*Santrauka*

*Alternaria* Ness. genties grybų sporų paplitimas ore tyrimo metais, sezono metu bei pasiskirstymas paros metu 2005–2006 metais tirtas trijose Lietuvos urbanistinėse vietovėse – Klaipėdoje, Šiauliuose ir Vilniuje. Nustatyti grybų sporų kiekiai per metus, sezono metu bei pasiskirstymas paros metu. Sporų fiksavimui naudota Hirsto tipo sporų gaudyklė. Grybų sporas buvo identifikuojamos ir skaičiuojamos 12 vertikalių juostų metodu. Grybų sporų sezono trukmė apskaičiuota taikant 90 % metodą.

Tyrimo metu sporų sezono trukmė buvo nuo 62 dienų Vilniuje 2005 metais iki 97 dienų Klaipėdoje 2006 metais. Gausiausi *Alternaria* sporų kiekiai buvo nustatyti vasarą, o mažiausi – žiemą bei pavasarį. Visose aerobiologinėse stotelėse pavienės sporos nustatytos vasario, kovo ir balandžio mėnesiais. Bendras *Alternaria* sporų kiekis per metus įvairavo nuo 3 090 sporų Vilniaus aerobiologinėje stotelėje 2005 metais iki 9 718 sporų Klaipėdoje 2006 metais. Sporifikacijos sezono pikai nustatyti rugpjūčio mėnesį visose tirtose vietovėse ir įvairavo nuo 121 iki 707 sporų m<sup>-3</sup>. Šį mėnesį buvo nustatyta 51,4–70,3 % sporų nuo metinio sporų kiekio. Paros metu gausiausios *Alternaria* sporų koncentracijos rugpjūčio mėnesį buvo nustatytos tarp 15:00 ir 1:00 valandos, mažiausios – 5:00–9:00 valandomis.

**Reikšminiai žodžiai:** *Alternaria*, grybų sporos, sezono trukmė, sporų koncentracija.

