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Poaceae Pollen Concentrations in the Atmosphere of Three Inland Croatian Sites (2003–2004)

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A B S T R A C T

The aim of the study was to determine the length of Poaceae *pollen season, intradiurnal, daily and monthly pollen variation, and the effect of some meteorological parameters on atmospheric pollen concentration, at three monitoring sites in inland Croatia during the 2003–2004 period. Seven-day Hirst volumetric pollen and spore traps were used for pollen sampling. At all three monitoring sites considerably higher precipitation and lower average temperature in 2004 led to a marked decrease in the grass pollen concentration in the air at all three monitoring sites. The highest grass pollen concentrations were recorded in Ivanić Grad (typical rural area), considerably lower in Samobor (effect of forest vegetation), and lowest in Zagreb (urban area). The highest atmospheric* Poaceae *pollen concentrations in inland Croatia were generally recorded in May and June. The highest intradiurnal concentrations were recorded between 8.00 and 12.00 a.m. Results of this aeropalynologic study are expected to help in preventing the symptoms of allergic reaction in individuals with* Poaceae *pollen hypersensitivity.*

Key words: *aerobiology,* Poaceae*, pollen concentrations, pollen allergy*

Introduction

The large *Poaceae* family presents a cosmopolitan distribution and represents almost 20% of the world vegetation cover. This family comprises about 9,000 species. They are cultivated for food (e.g. cereals, corn, rice, sugar cane) and animal forage, kept as managed surface vegetation on recreational areas, and as turf to prevent soil erosion.

Contributing to the competitive advantage and versatility of grasses is the location of the leaf meristem at the base blade, an arrangement which allows continued growth after grazing or other forms of damage to the leaf, such as fire or drought. Most grass species are wind pollinated and produce large amounts of light, airborne pollen during a short period ranging from a few hours to a few days, although some species develop new flowers continuously[1–3]. Another situation concerns a family of different species with the same allergenic properties, thus prolonging the period of time during which allergenic pollen can be found in the air. Given all the above-mentioned characteristics, this family is one of the major causes of pollen allergies in the world. Twenty per cent of allergic patients in Denmark suffer from grass pollen allergy. In Holland and France 80% of allergic patients suffer from grass pollen allergies[4]. In recent years, several papers have highlighted the differences or similarities that exist between pollen counts in the atmospheres of several specific locations in regions with different climates and relationships that exist between meteorological parameters and atmospheric pollen concentrations[5–7].

The first research into allergenic pollen distribution in Croatia was launched in 1959 at four locations: Zagreb, Hvar, Crikvenica and Dubrovnik[8]. Using gravimetric method, data were collected weekly throughout the year. Since 1973, allergenic pollen has been continuously studied in Zagreb[9]. Modern aerobiologic investigations using daily volumetric monitoring of atmospheric pollen began in Croatia in 2002[10].

The aim of the study was to determine the length of pollen season, intradiurnal, daily and monthly pollen variation, effect of some weather parameters on atmospheric pollen concentration, and possible variation in the occurrence and concentration of *Poaceae* pollen grains at three monitoring sites in inland Croatia during the 2003–2004 period. Results of this aeropalynologic study are expected to help in preventing the symptoms of allergic reaction in individuals with *Poaceae* pollen hypersensitivity, thus improving their quality of life.

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Materials and Methods

Measuring sites

The study was carried out at three inland sites in Croatia during two years (2003–2004). The samplers were placed in Zagreb, Samobor and Ivanić Grad. These locations differed in terms of their geographic location and topography (Table 1).

Zagreb is situated at the foothills of the Medvednica mountain, and the composition of airborne pollen in the City of Zagreb is strongly influenced by the forest vegetation from the north, consisting of the following species: *Acer* sp., *Ulmus glabra* Hudson, *Fraxinus excelsior* L., *Carpinus betulus* L., *Ostrya carpinifolia* L., *Corylus avellana* L., *Fagus silvatica, Quercus petraea* (Mattuschka) Liebl., *Taxus baccata* L., *Abies alba* L. (at higher altitudes), *Castanea sativa* L., various species of the genus Rubus, *Sambucus nigra* L., *Robinia pseudoaccacia* L., etc. Grassy and weedy grounds prevail eastward and soutward of the city. In these areas, the vegetation consists of a number of ruderal and weedy, wild-growing and adventive plants, primarily from the families *Poaceae*, *Chenopodiaceae, Compositae, Brassicaceae, Urticaceae, Lamiaceae* and *Fabaceae*. Of adventive plants, the allergenic species *Ambrosia artemisiifolia* L. and *Artemisia vulgaris* L. are most widespread. In the urban area, along the Sava river, the species *Alnus glutinosa* (L.) Gaertn. and various species of the genera *Salix* and *Populus* prevail. In addition to this more or less natural vegetation, the city has numerous parks containing the following species: *Betula pendula* L., various species of the family *Cupressaceae* and of the genera *Pinus* and *Picea, Aesculus hippocastanum* L., *Platanus hybridus, Populus* sp., *Juglans regia* L., *Tilia* sp.

Ivanić Grad is most rural of the three monitoring locations, with a lot of weedy areas. The respective airborne pollen also showed some elements of typical inland forest vegetation. Great impact of ruderal and weedy vegetation was recorded, with numerous species of the families *Brassicaceae, Asteraceae, Amarantaceae, Apiaceae, Plantaginaceae, Poaceae,* etc. The cultivation also contained some taxa from the family *Cupressaceae*, then *Betula pendula, Taxus baccata, Pinus, Picea and Abies sp., Tilia sp.,* etc.

The region of Samobor is surrounded by the Žumberak-Samobor mountains with large forests around the town and with numerous autochthonous and planted trees also found in the town. Airborne pollen is influenced by this forest vegetation, which is predominated by many forest species such as *Acer campestre, A. pseudoplatanus, A. platanoides, Carpinus betulus, Corylus avellana, Fagus silvatica, Quercus petraea, Ulmus glabra, Castanea sativa, Taxus baccata,* etc. In addition, this vegetation also includes a number of species characteristics of sub-Mediterranean climate. Such a type of vegetation is mostly found on the south mountain slopes and includes woody plants such as *Quercus pubescens, Ostrya carpinifolia* (more common than *Carpinus betulus*) and *Fraxinus ornus*. A small river is running through the town, favoring the growth of woody plants characteristic of humid habitats, with a predominance of the species *Alnus glutinosa* and various species of the genera *Populus* (mostly *P. alba* in lower areas, and *P. tremula* in higher areas) and *Salix* (mostly *S. caprea*). Ruderal and weedy vegetation is less abundant than in the Zagreb area, and is predominated by adventive taxa of the families *Asteraceae* and *Poaceae*. In the city parks and gardens there is a great variety of different taxa (autochthonous and horticultural) of the genera *Pinus, Juniperus/Cupressus, Abies* and *Picea*, then widely planted *Betula pendula, Juglans regia* and *Tilia* sp.

Pollen sampling and counts

Seven-day Hirst volumetric pollen and spore traps were used for pollen sampling[11,12].

The sampler absorbs 10 L air *per* min. It is supplied with a timer to move the adhesive tape (2 mm/h) to which pollen grains adhere. The tape was removed twice weekly, cut to a length corresponding to 24-h pollen sampling applied onto glass slide and embeded in the following medium: 70 g polyvinyl alcohol (Gelvatol) and 4 g phenol (C_6H_6O), and dissolved in 200 mL of distilled water. After overnight rest, 100 mL glycerol ($C_3H_8O_3$) were added and warmed up in water bath until the solution turned liquid and clear. Then, 4 drops of alcohol solution of basic fuchsin ($C_{20}H_{20}ClN_3$) *per* 100 mL were added. Samples were examined under a light microscope, magnification x400, to determine pollen type and count *per* 1 m^3 air *per* day. Five horizontal sweeps on each slide were analyzed. We have decided to choose horizontal sweeps, because the variation of the concentration during the day can be observed along this axis (direction of the shift of the tape in the sampler). The minimum number of horizontal sweeps is the one that assures a total observed area that is at least 20% of the sampled area. The accuracy of the measurement is proportional to the number of sweeps and to the concentration of particles[13]. Pollen concentration was expres-

TABLE 1
SAMPLING SITE CHARACTERISTICS

Site	Geographic position	Altitude (m)	Location of sampler (m above ground level)	Site size
Zagreb	45° 49' N, 15° 59' E (in the centre)	157	19.7	about 1,000,000 inhabitants
Samobor	45° 48' N, 15° 43' E (in the centre)	168	18.5	about 36,000 inhabitants
Ivanić Grad	45° 43' N, 16° 24' E (in the centre)	101	17.3	about 15,000 inhabitants

sed as pollen grain count/m^3 air and characterized as absent, low, moderate, high, or very high[14]. The meteorological data for each location were provided by different stations of the Croatian Weather Bureau (Zagreb-Grič, Čazma and Samobor). The meteorological variables used in the study were average temperature and precipitation (Figure 1, Table 2).

In order to define the main pollen period, the study was limited by eliminating days with minimal pollen concentrations at the start and end of pollen season. These non-estimated periods accounted for 2.5% of total pollen concentration, both at the start and at the end of pollen season[15]. Pollen data set in relation to the meteorological parameters was tested with Spearman's rank test.

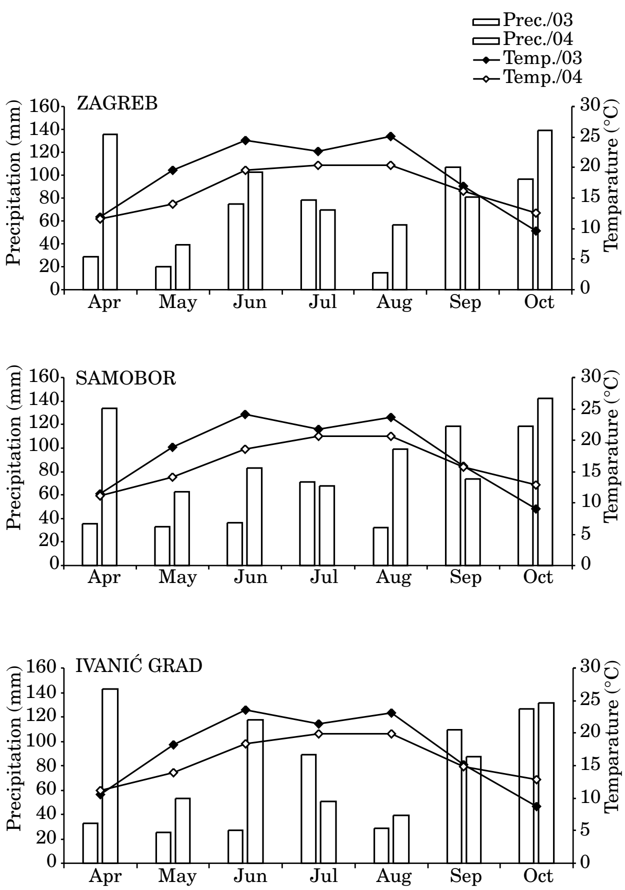

Fig. 1. Meteorological conditions during Poaceae *pollen seasons at monitoring sites in Zagreb, Samobor and Ivanić Grad 2003–2004.*

TABLE 2
MEAN YEARLY AIR TEMPERATURES AND PRECIPITATIONS COMPARED TO THE LONG-TERM MEANS

Meteorological station	Temperature (°C)			Precipitation (mm)		
	Long-term average (1961–1990)	2003	2004	Long-term average (1961–1990)	2003	2004
Zagreb	11.2	12.9	11.2	882.8	624.4	918.4
Samobor*	10.4	11.7	11.2	1181.9	728.4	1044.6
Čazma	10.5	11.4	10.9	880.3	692.0	972.0

*long-term average for Samobor (1972–1990)

Results

The aeropalynologic studies conducted during the 2003–2004 period showed the total annual grass pollen concentration to be higher in 2003 than in 2004 pollen season at all monitoring sites, i.e. by 49% in Samobor, and by 56% in Zagreb and Ivanić Grad. In both study years, the highest airborne grass pollen concentration was measured at the monitoring site in Ivanić Grad (n_{03}=5,730, n_{04}=2,529), considerably lower in Samobor (n_{03}=2,918, n_{04}=1,495) and even lower in Zagreb (n_{03}=2,711, n_{04}=1,201) (Figure 2). The length of pollen season varied according to both monitoring site and year of observation, being longest in Zagreb in 2004 (171 days, April 18–October 5), and shortest in Ivanić Grad in 2003 (146 days, April 16–September 8). The peak day was recorded in the second half of May at all monitoring sites except for Samobor, where it occurred on June 2nd (Table 3).

The highest atmospheric pollen concentrations in inland Croatia were generally detected in May and June. In these months, the percentage of particular grass pollen types in total monthly pollen concentration of all taxa ranged from 14.8% in Samobor (2004) through 53.0% in Ivanić Grad (2003). At all monitoring sites, they were below 1% in April, and did not exceed 10% in other months (Figure 3).

At all monitoring sites, grass pollen concentrations were significantly influenced by temperature and precipitation. Comparison between 2003 and 2004 revealed a considerably higher level of precipitation accompanied by lower average temperature in all months of pollen season in 2004. The level of rain recorded in April 2004 was by 74%–79% greater than that observed in the same month of 2003. This trend continued in May and June, whereas the level of rain recorded in July and September 2004 was lower from the figures for 2003 at all monitoring sites. The greatest differences in the mean daily temperature between 2003 and 2004 were recorded for the month of May, showing a clear decline by 24%–29% in 2004 ($temp_{03/04}$=19.6°C/14.1°C in Zag-

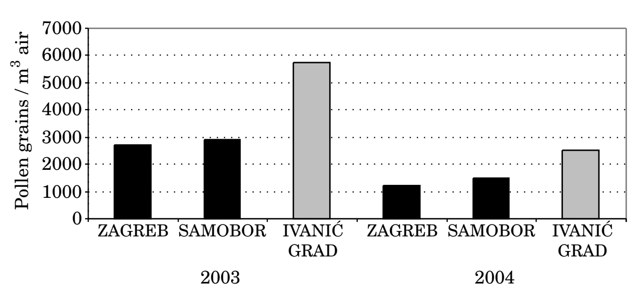

Fig. 2. Annual Poaceae *pollen concentration at sampling sites in Zagreb, Samobor and Ivanić Grad.*

TABLE 3
DATA ON THE PRESENCE OF *POACEAE* POLLEN AT THREE MONITORING SITES, 2003–2004

Site	Year	Period of occurrence	Range (days)	Peak day	Peak day concentration (pollen grains/m^3)
Zagreb	2003	April 20th–September 23rd	157	May 19th	154
	2004	April 18th–October 5th	171	May 31st	81
Samobor	2003	April 27th–September 22nd	149	May 27th	276
	2004	April 22nd–October 2nd	164	Jun 2nd	194
Ivanić Grad	2003	April 16th–September 8th	146	May 28th	512
	2004	April 15th–September 20th	159	May 31st	312

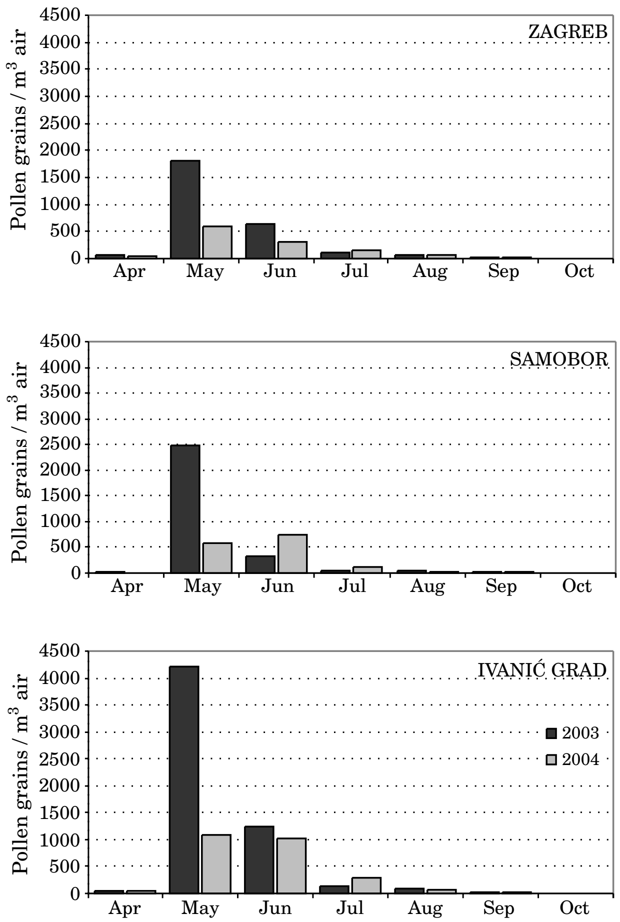

Fig. 3. Monthly variation in Poaceae *pollen concentration at monitoring sites in Zagreb, Samobor and Ivanić Grad 2003–2004.*

TABLE 4
SUMMARIZED RESULTS OF THE STATISTICAL ANALYSIS POLLEN DATA IN RELATION TO THE METEOROLOGICAL PARAMETERS

Pair of variables	Spearman rank order correlations			
	Valid N	Spearman rank	t(N-2)	p-level
2003				
Temperature and pollen				
Zagreb	61	0.442981	3.795302	0.000350*
Samobor	61	0.147716	1.147210	0.255926
Ivanić Grad	61	0.380160	3.157096	0.002511*
2004				
Precipitation and pollen				
Zagreb	61	–0.286531	–2.297210	0.025172*
Samobor	61	–0.451065	–2.674330	0.012358*
Ivanić Grad	61	–0.464095	–2.772410	0.009783*

Marked values (*) are statisticallysignificant (p<0.05), pair of variables – variables in correlation, Valid N – valid number of variables, t (N-2) – t distribution on N-2 degrees of freedom, p – level – probability level

reb; $temp_{03/04}$=18.9°C/14.1°C in Samobor; and $temp_{03/04}$= 18.2 °C/13.9 °C in Ivanić Grad). Such weather conditions resulted in considerably lower airborne pollen concentrations at all monitoring sites in 2004 (Figures 1 and 3). Finally, using Spearman's rank test we found correlations between higher air temperature and high pollen concentrations as well as high precipitation and low pollen concentrations (Table 4).

Small differences were observed in the intradiurnal pollen distribution according to monitoring sites. In May and June of both study years, an increase in the airborne pollen concentration was recorded in the early morning, between 4.00 and 6.00 a.m., followed by a decline, to rise abruptly again over a 6.00 to 8.00 a.m. lapse of time. In Zagreb and Samobor, the highest daily concentrations were measured between 10.00 and 12.00 a.m., when daily temperature increased and relative humidity decreased. In Ivanić Grad, the highest daily concentrations were recorded between 8.00 and 10.00 a.m. The intradiurnal air temperature increase was closely accompanied by pollen concentration increase and the relative humidity decline (Figures 4 and 5, Table 5).

Discussion

Comparison of total annual grass pollen concentrations in Zagreb (n_{03}=2,711, n_{04}=1,201) with those reported from some European cities shows comparable figures for Copenhagen, and considerably higher figures for Cardiff (n=7,400), Leiden, Brussels, Munich and Na-

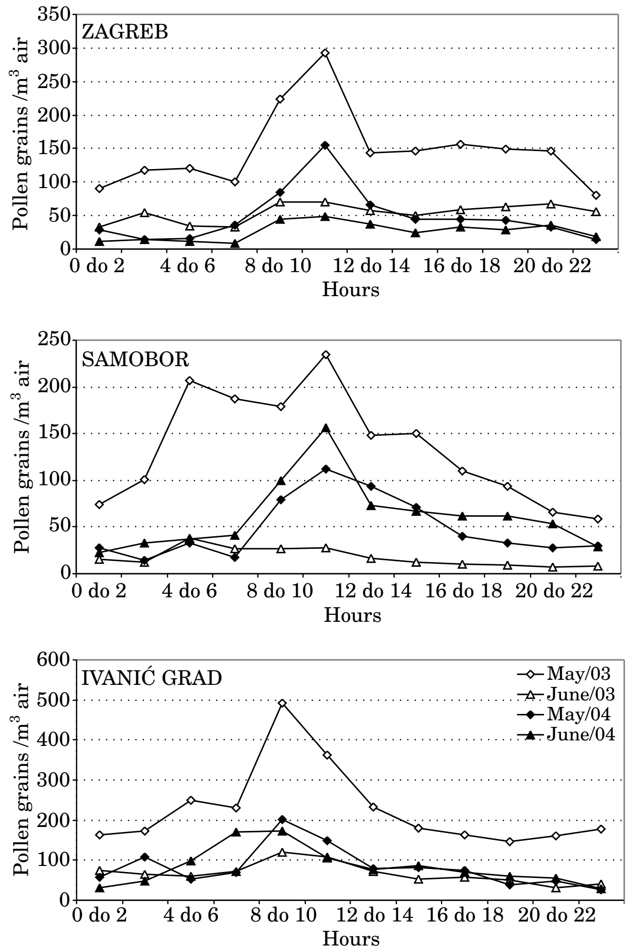

Fig. 4. Intradiurnal Poaceae *pollen concentration at monitoring sites in Zagreb, Samobor and Ivanić Grad in May/June 2003–2004.*

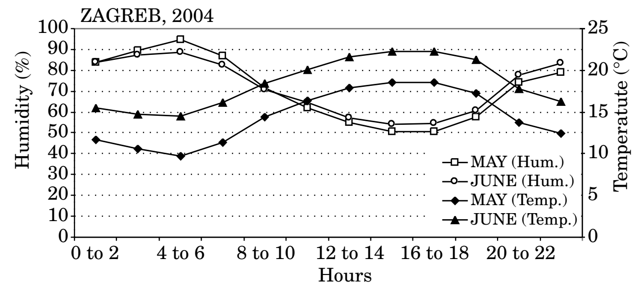

Fig. 5. Intradiurnal temperature and relative humidity variation in May/June 2004.

TABLE 5
RESULTS OF THE STATISTICAL ANALYSIS INTRADIURNAL POLLEN DATA IN RELATION TO THE TEMPERATURE AND HUMIDITY

Pair of variables	Spearman rank order correlations			
	Valid N	Spearman rank	t(N-2)	p-level
May – Temperature and pollen	12	0.715789	3.24140	0.008848*
May – Humidity and pollen	12	–0.711034	–3.19770	0.009529*
June – Temperature and pollen	12	0.630474	2.56855	0.027960*
June – Humidity and pollen	12	–0.602453	–2.38691	0.038159*

marked values (*) are statisticallysignificant ($p<0.05$), pair of variables – variables in correlation, Valid N – Valid number of variables, t (N-2) – t distribution on N-2 degrees of freedom, p – level – probability level

ples (n=3,000). The highest seasonal totals were observed in Cardiff and lowest in Copenhagen. The seasonal total in inhabited environments is about 4,000–5,000 grass pollen grains/m^3 outdoor air[4]. These data rank Zagreb among cities with lowest grass pollen concentrations in Europe. Ivanić Grad as a typically lowland rural area surrounded by uncut grass showed highest total annual grass pollen grain counts in the air, yet below those reported from Cardiff.

The grass pollen season of 146–171 days in inland Croatia is somewhat longer than in the above mentioned European cities, exceeding the values reported from these as well as from some cities in Spain that were not included in the above cited study, i.e. Leon, Cordoba and Santiago de Compostela (92, 93 and 94 days, respectively). This period is only longer in Malaga and Barcelona, ranging from 271 to 276 days. In inland Croatia, the peak of pollen season was observed in the second half of May, like other south European countries. In both study years, the onset of pollen season was recorded towards the end of April at all monitoring sites, which could be explained by meteorological patterns that did not differ substantially across the relatively small study area. In inland Croatia, the beginning of grass pollen season occurred by 15–30 days earlier than in southeast Poland. At that time, air temperature was slightly above 11°C in both study years, which is consistent with the data reported from Vigo and Santiago de Compostela[4,15,16]. These data are highly important, because temperature and precipitation are the main control factors at the start of the grass pollen season and also the moment when peak values are reached[17,18]. Differences in temperature and precipitation between 2003 and 2004 influenced pollen concentration throughout the pollen season. High precipitation in 2004, especially at the beginning of pollen season, and lower temperature led to a significant decrease in the concentration of grass pollen in the atmosphere. The aerobiological graphics of *Poaceae* is characterized by long periods of low pollen concentration and one relatively short period of maximal pollen production (between 6 and 8 weeks). This has already been reported for locations in the Mediterranean and Atlantic Europe[4,7,19]. Examination of diurnal variation in *Poaceae* pollen shows how the average pollen concentration (regardless of differences in temperature and humidity) achieves maximum between

10.00 and 12.00 a.m. The highest pollen concentrations were recorded at daily temperatures above 20°C. In our study, grass pollen concentration was minimal between 6.00 and 8.00 a.m., to rise then gradually during the day, a pattern also recorded in London[20]. However, there is variation in the size of peak concentrations and slight difference in timing with temperature. The difference is only obvious in maximal airborne pollen concentration, which in London occurs at about 8.00 p.m. This difference in timing between our and London results from the fact that the grass pollen measured at our monitoring sites originated from the immediate sampling site surrounding, whereas the majority of grass pollen measured in London originated from the city outskirts. The delay observed in London was related to wind speed, wind direction, and pollen source location[21].

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KONCENTRACIJE PELUDA TRAVA U ZRAKU KONTINENTALNE HRVATSKE (2003–2004)

S A Ž E T A K

Cilj rada bio je odrediti duljinu peludne sezone biljaka iz porodice trava, intradiurnalne, dnevne i mjesečne varijacije koncentracija peluda te utjecaj nekih meteoroloških parametara na koncentraciju peluda trava u zraku kontinentalne Hrvatske u razdoblju od 2003.–2004. godine. Za uzorkovanje se koristio sedmodnevni volumetrijski uzorkivač tipa Hirst. Na svim mjernim postajama u 2004. godini primijećen je pad koncentracije peluda u zraku zbog izrazito veće količine padalina i nižih temperatura u odnosu na 2003. godinu. Najviše koncentracije peluda u zraku zabilježene su u Ivanić Gradu (tipično ruralno područje), bitno niže u Samoboru (utjecaj termofilne šumske vegetacije), te najniže u Zagrebu (urbana sredina). Vrhunac peludne sezone na svim mjernim postajama bio je u mjesecu svibnju i lipnju. Najviše intradiurnalne koncentracije zabilježene su između 8:00 i 12:00 sati. Rezultati ovog rada predstavljaju pomoć u prevenciji pojave simptoma alergijske reakcije u osoba alergičnih na pelud trava.