Abstract: The aim of the present study is to compare the pollinic season of Plantago in four aerobiological stations within the Danube-Kris-Mures-Tisza euroregion. The Plantago pollen has generally been considered a minor cause of pollinosis. It is difficult to evaluate the role of Plantago airborne pollen in the pollinosis symptomatology because of the low rate of monosensitive patients. Moreover, the allergy to Plantago airpollen is connected with the allergy to Poaceae pollen because of the simultaneous presence of the two types of pollen in the air. Since the onset of the allergy also depends on the airpollen concentration, in this study we intend to present the concentrations of the Plantago pollinic type within the Danube-Kris-Mures-Tisza euroregion. The analysis uses the data obtained during five years of monitoring with the help of four volumetric traps located in Timişoara (Romania), Szeged (Hungary), Novi Sad and Ruma (Serbia). The Plantago pollen is present in the air from May until August. The highest total annual concentration was recorded at Novi Sad in 2001 (1326 pg/m³). The highest daily airpollen concentrations seldom exceed 30 pg/m³. The highest daily concentration was 64 pg/m³ (recorded in Novi Sad, 2001). In Szeged and Novi Sad the annual concentrations are on the decrease, while in Timişoara they are slightly on the increase. The presence of the pollen in the airplankton was considerably long in Timişoara, Novi Sad and Ruma in 2004.

Key words: airborne pollen, airplankton of cities, Plantago

Introduction

Several authors have already reported that Plantago lanceolata (English plantain, ribwort) belong to the most important pollens and should therefore be included in the test spectrum for allergological examinations. Allergic sensitization to Plantago pollen is fairly common. It was first reported by Bernton (1925) and there is a relatively large bibliography available on the subject; numerous researchers have carried out studies on this pollen type, including Tuft & Blumstein (1937), Serafini (1957), Duchaine & Spapen (1961), Charpin et al. (1962), Izco et al. (1972), Lewis (1977), Saenz de Rivas (1978), Bousquet et al. (1984), Subiza Martin et al. (1986), Watson & Constable (1991).

Few studies have tried to identify the allergens in Plantago pollen. In 1980, Baldo et al. detected at least six IgE-binding allergens in the molecular weight range of 10–300 kDa. More recently, Dreborg et al. found at least 13 allergens by immunoblotting with one component of 15 kDa with a high degree of specific IgE binding. Only a 30 kDa plantain allergen cross-reactive with the grass group 5 allergens has been identified to date, yet this cross-reactivity shows little or no clinical relevance, as suggested by Asero et al. (2004).
These authors also reported in the same article that IgE from monosensitized plantain-allergic patients mainly reacted with 17, 19 and 40 kDa allergens. Calabozo et al. found that the 17 and 20 kDa allergens are the unglycosylated and glycosylated forms of the same protein (Pla l 1) respectively, and that the 32–36 kDa protein is a dimeric form of the same Pla l 1 allergen. Moreover, the major complex N-glycan of Pla l 1 might be a potential source of cross-reactivity with other glycosylated pollen allergens that could be misleading in terms of false positive diagnoses of allergy to plantain when using natural extracts. Therefore the generation of a recombinant Pla l 1 without the complex N-glycan as part of its structure would be a very useful tool to diagnose patients specifically sensitized to plantain. The glycoprotein Pla l 1 is the major allergen from Plantago lanceolata pollen, which is a common cause of pollinosis in temperate areas. The major allergen of Olea europaea pollen has been found to share sequence similarity to Pla l 1[10].

**Material and methods**

The aerobiological monitoring concerning the pollen content of the airplankton was carried out by using the volumetric method of collecting data. This is the method that most researchers use for qualitative and quantitative studies of airborne pollen and fungi. It implies the repeated succession of two phenomena: the absorption of a constant volume of air and the immediate trapping of the airborne particles as they impact a trapping surface.

The traps used were of the Hirst type, model VPPS 2000, Lanzoni. The trap allows for an evaluation of the dynamics of the allergenic atmospheric pollen in the town/city and its surroundings. In order to regularly collect the data and to get correct statistics, the traps are placed at locations higher than 20 meters, far from industrial areas and barriers which might prevent the circulation of the air currents. The bands inside the volumetric traps were changed weekly.

Pollen identification was carried out according to morphological criteria [23, 46]. The pollen concentration is expressed in number of pollen grains per m³ of air. Our bulletins were published weekly, from February until October on the following websites: Euroregional Polleninformation Service Danube-Kris-Mures-Tisza Euroregion (www.pollinfo.int.hu) and www.nspolen.com. The DKMT Euroregion includes the following counties: Bács Kiskun, Csongrád, Jász-Nagykun-Szolnok, Békés (Hungary), Arad, Timiș, Hunedoara, Caraș Severin (Romania), and Vojvodina (Serbia).
Results and discussions

The main pollen types with a role in allergic sensitization come from anemophilous plants. The pollination duration matches the symptomatology of clinic manifestations. Spieksma (1991) included Plantago pollen into sporomorphs revealing high level of allergenicity, and these taxa were placed on the list comprising the most important plants which should be considered in pollen monitoring in European research centres.

In 2000, the highest total annual concentration was recorded in Szeged (472 PG/m³). In Timişoara the concentration reached only half of the result recorded in Szeged, representing 1.72% of the pollinic range of the year 2000.

In 2001, the Novi Sad station reported a total concentration of 1326 PG/m³. In Timişoara, the concentration of Plantago pollen (148 PG/m³) diminished as compared to the concentration recorded the previous year and represented a mere 0.94% of the annual pollinic range. In 2002, the airpollen concentrations recorded in Novi Sad and Timişoara were relatively similar: 797 PG/m³ and 669 PG/m³. In Timişoara, the concentration represented 3.28% of the total annual of 20068 PG/m³. In 2003, the Ruma monitoring station started recording data in Serbia, alongside the Novi Sad station. The total annual concentration was highest in Timişoara, representing 2.67% of the total annual of 24557 PG/m³. In 2004, the highest concentration (486 PG/m³) was recorded in Timişoara. As to the interannual variation (fig.2), the linear regression model shows the decreasing trend in the annual concentrations for Szeged and Novi Sad, while an increasing trend can be noticed for Timişoara. We can state that in the DKMT Euroregion the Plantago airpollen is a constant presence in the pollinic range, yet its quantity is moderate. This situation is also present in other parts of Europe, where the annual concentrations do not exceed 10% [20]. In Europe, this pollinic type proved to be dominant in Montpellier, France [43], Athens [1], Bitlis, Turkey [12], London, Leiden, Brussels, Munich, Marseille [41], Madrid [44], Salamanca [22], Northwestern Spain [36], Estepona, Southern Spain [35], Belgium [42, 43].
The longest period (8 days) when the number of pollen grains exceeded 30 PG/m³ was reported in Novi Sad in 2001. Another two values over 30 PG/m³ were also registered in Novi Sad: one in 2000 and the second, in 2002. In Szeged and Ruma the threshold value was never exceeded. In Timișoara, the sensitization threshold value was exceeded for three days in 2003 and one day in 2002. The stations in the Euroregion did not register excessive values in 2004. The highest daily concentration (fig.3), 64 PG/m³, was registered in Novi Sad in 2001. For Szeged the highest value (22 PG/m³) was registered in 2000; for Timișoara the highest value (42 PG/m³) was registered in 2003. These concentrations are exceptions from the usual values throughout the pollinic season. Similar situations were recorded in Spain [20], Poland [50], Turkey [7, 21], Hungary [26], and Croatia [32].

The *Plantago* species pollinate from May until the end of August. By considering the data which refer to the number of days when the pollen was present in the airplankton, very wide variations were noted: 97 to 107 days in Szeged, 108 to 137 days in Novi Sad, 102 to 138 days in Ruma, and 76 to 136 days in Timișoara. In 2004, the presence of the *Plantago* airpollen until the first decade of September did not correlate with an increase in the daily or annual concentrations. In this paper, we determined the APS (Atmospheric Pollen Season) in accordance with the criteria used by the following authors: Nilsson and Persson (corresponding to 90% of the total pollen catch -the 90% method), Andersen and Torben (corresponding to 95% of the total pollen catch- the 95% method) [25]. The longest pollinic season was that in Ruma and the shortest, in Szeged (tab.1). The duration of the pollinic season and the total annual airpollen concentration were relatively close in Timișoara and Novi Sad. Similar variations were recorded in Poland: 95 to 105 days in Rzeszów, 69 to 92 days in Krasne [28], 62 to 98 days in Lublin [50]. In Ankara, *Plantago* was included in the pollinic group with a maximum pollinating period longer than 15 weeks [27].

Fig.2. Dynamics of annual concentrations of *Plantago* airpollen (2000-2004)
Several authors report that between 3%-36% of patients are allergic to Plantago, most being polysensitized and, therefore, also allergic to the pollen of other plants, mainly Poaceae. Most patients positive to the skin prick test (SPT) with plantain-pollen extracts are hypersensitive to Poaceae [6, 49; 38], a fact which suggests that at least one allergen in grass and plantain pollens cross-reacts. In a series of 242 consecutive grass-pollen-allergic patients, 71 (29%) were positive in the SPT with plantain-pollen extract.

The results suggest the existence of common antigenic epitopes in melon and Plantago pollen, and in melon and grass pollen [18]. The pollen contains epitopes made up of major and minor determining (antigenic, allergenic) groups of amino acids. An individual may be sensitized either to several major and minor epitopes or to a single epitope, be it a minor one. Cross-reactivity phenomena may occur especially because of homology, but also because of the structural mimocrimy of some epitopes, both within the
same species and between different species. The cross-reactivity phenomenon may be produced not only by various pollen types, but also by food [34]. In other countries, a relationship between changes in crops and variation in pollen sensitization has been observed [38].

Subiza et al. (1995) reported an average airborne Plantago pollen count in Madrid of 3.6%, with positive skin tests of 53% to P. lagopus pollen, 32% to P. lanceolata and 55% to P. lagopus and/or P. lanceolata. Garcia Gonzales (1995) in Málaga reported that 8% of patients were allergic to P. lanceolata. Recently, several articles have yielded clinical results from tests carried out in several Spanish cities, with sensitization percentages varying between 15% for Málaga [47]) and 78.24% for Toledo [30]. Regarding patient sensitization, sensitivity was detected in Thessaloniki - Greece to plantain in 194 patients (14.6%) [19].

In the period of plantain pollination, Bryant et al. reported from Sydney that the patients developing asthma symptoms were simultaneously allergic to the plantain allergens. The allergy to plantain allergens was noted in 84% patients with asthma [8]. Of the 629 patients, 459 gave positive SPT results to at least one pollen. No statistical differences were found with respect to gender, habitat (rural or urban) and age.

Sensitizations to the different botanical families were as follows: 384 patients were sensitized to Poaceae family pollen, 348 patients to the Oleaceae family, 249 patients to the Plantaginaceae family, 211 patients to the Chenopodiaceae family, 153 patients to the Cupressaceae family, 94 patients to the Platanaceae family, 94 patients to the Compositae family, 80 patients to the Betulaceae family and 27 patients to the Urticaceae pollen. Multiple Correspondence Analysis proved the existence of associations among pollen sensitizations, showing that they clustered into two groups: Group I which included Poaceae, Oleaceae, Cupressaceae, Chenopodiaceae and Plantaginaceae and Group II, which included Betulaceae, Platanaceae and Compositae. Pollens of the association Group I do not coincide with those collected in largest numbers in the Madrid atmosphere, since the total annual pollen grain count is highest for Poaceae, followed by Cupressaceae, Platanus, Olea and Plantago [44, 4]. Two distinct behaviors could be observed in Milan: a) a high propensity to develop new respiratory allergies characterized patients allergic to Poaceae (46%), Parietaria (35%), and Betula pollen (37%) whereas b) patients allergic to house dust mite (15%), Ambrosia (15%), Alternaria (11%), Artemisia (22%), and Plantago (20%) showed a much lower propensity to develop new allergies. The “new” allergens (Ambrosia and Betula) caused 228/256 (89%) new sensitizations detected in the whole study group, included patients allergic to Plantago [2].

It has been observed that is not a clear relationship between the amount of pollen collected in the air and the incidence in allergy people [38]. Percentage of patients displaying reactions to Plantago pollen type according to SPT was 13.33% in Cordoba and 21.42% in Evora. Sum of daily pollen counts during the study period was 606 in Cordoba and 184 in Evora. Result of correlation between Plantago pollen counts and symptoms suggest a lower incidence of allergic diseases related to pollen in the city of Evora. This fact could be explained by two main causes.

Firstly, 73.34 % of patients in the city of Cordoba were aged between 11 and 30 years old, whereas only 50% of patients were included in group. A study performed across
Spain reported that the 14-25 age-group was the most affected by pollinosis. Secondly, the population in Evora has maintained a rural lifestyle for a long time whereas in Cordoba people have changed to an urban lifestyle along the last decades.

Some studies suggest that acquisition of certain infections or exposure to naturally occurring microbial exposures as encountered in the rural environment could confer protection from allergic diseases. The atmosphere in Evora is by far less contaminated than in Cordoba where the main source of solid material emissions into the air is road traffic, since the city lies on the route for goods transported from the southern to the central regions of the country. Córdoba has recently seen a reduction in the number of ecosystems where grasses are able to grow, due to the expansion of the city and to town-planning changes [38, 11]. Prevalence of allergic reactions to *Plantago* pollen type in each area was 9.24 in North, 10.15 in West, 11.21 in South, 6.05 in Centre, 12.52 in East. Percentage of patients displaying reactions to *Plantago* pollen type was: 29.16% (between 1984 -1990) and 33.87% (between 1999 -2001). A positive and significant correlation was observed between monthly pollen indices and antihistamine sales for *Plantago* [38].

In the sample of population of Sarajevo region during the 2002 year has been investigated on the pollen of weed plant species and *Poaceae* pollen. In the mixture of pollen weed plant species have been following plants: *Plantago lanceolata*, *Chenopodium album*, *Solidago gigantea*, *Artemisia vulgaris* and *Urtica dioica*. 589 have been tested patients by mixture of pollen mentioned plant species and found 115 as a sensitive on pollen alergy; 65 male and 50 females. Even 61 are children to 14 years, or 53% of total sick patients [39].

In Romania, hypersensitization to the mixture of pollen coming from grasses (*Artemisia vulgaris*, *Plantago lanceolata*, *Rumex acetosella*, *Urtica dioica*) was found in 13.13% of the cases [33] and 2.77% of the patients [16].

A study carried out in France points out that some of the children suffering from atopic dermatitis (9.8%) are also sensitive to *Plantago* aeroallergens [9].

The number of people allergic to plant aeroallergens has substantially increased in big cities and industrial areas [31]. Thus, monitoring of the pollen counts in the airplankton of cities is of relevant medical importance.

The concentration of pollen grains in the air over a city is determined by the individual rhythm of plant pollination, meteorological conditions, composition of local flora, geographic location and kind of urban structure (loose or compact housing, areas with many gardens or with scarce vegetation, industrial areas, agricultural areas or forests) [51]. The higher temperatures in a town can cause a longer vegetative period.

The microclimate of towns is characterized by reduced levels of relative air humidity, specific winds, an increased content of aerosols in the air, and a greater frequency of fogs. The generally accepted conclusion is that the participation of arboreal pollen in the pollen fall reflects regional conditions, while the content of pollen of herbaceous plants reflects local ones [20].

Results of this study demonstrate that *Plantago* seasons occurred at regular intervals between May and August each year; however, individual daily and seasonal *Plantago* counts were heterogeneous. The start of APS (Atmospheric Pollen Season) was relatively constant for *Plantago*, while the end of APS showed significant variations. Registered data
confirm the fact that at least quantitatively *Plantago* pollen is not an important allergenic factor. Overall, the pollen shedding course of the *Plantago* in Danube-Kris-Mures-Tisza Euroregion corresponds to that already described during the pollen season in other European areas [23].

**Conclusions**

In the DKMT Euroregion the *Plantago* airpollen is a constant presence in the pollinic range, but it is moderately represented from a quantitative point of view.

The highest annual concentration (1326 PG/m$^3$) was recorded in Novi Sad in 2001, while the lowest annual concentration (134 PG/m$^3$) was recorded in Ruma in 2004.

In Szeged and Novi Sad the annual concentrations are on the decrease, while in Timișoara they are slightly on the increase.

The highest daily concentration (64 PG/m$^3$) was recorded in Novi Sad in 2001.

The threshold value (30 PG/m$^3$/day) was seldom exceeded.

There is no correlation between the longer presence in the airplankton and an increase in the concentrations.

**REFERENCES**

5. BERNTON H. S., 1925. Plantain hay-fever and asthma. JAMA, 84: 944-946
27. KAPLAN A., 2004 - Predominant aeroallergen pollen grains in the atmosphere of Ankara, Turkey, Allergy, 59: 670–672
28. KASPRZYK I., 2006 - Comparative study of seasonal and intradiurnal variation of airborne herbaceous pollen in urban and rural areas, Aerobiologia, 22:185–195
40. SERAFINI U., 1957 - Studies on hay fever (with special regard to pollinosis due to Parietaria officinalis). Acta Allergolog. 11:3-27
48. TUFT L., BLUMSTEIN G. L., 1937 - Incidence and importance of tree pollen hayfever with particular reference to Philadelphia and vicinity. J. Allergy, 8: 464