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THE PRINCIPAL AIRBORNE AND ALLERGENIC POLLEN SPECIES IN TIMIŞOARA

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ABSTRACT
The most predominant source of allergens in the outdoor environment is pollen, the male gametophyte of flowering plants. Allergenic pollen has been identified in many flowering plant species including grass, weed, tree and crop species. Occurrence of pollens is monitored almost throughout Europe. We report here the results of the monitoring of airborne pollen concentrations throughout 1999 to 2007 from Timişoara (România). A total of 18 allergenic pollen types were identified of which Ambrosia artemisiifolia pollen showed maximum concentration. Pollens of many plants located in public or private gardens may cause pollinosis in predisposed individuals. In this study we suggest a list of recommended plants for public and private green.
KEY WORDS: *pollinosis, aeroallergen, wind-pollinating species*

INTRODUCTION

Allergen molecules are present in indoor and outdoor environments in many forms. Allergens are commonly proteins or glycoproteins (Aas, 1978). A protein is defined as a major allergen when it causes an allergic reaction in at least 50% of individuals sensitive to the particular species from which the allergen is derived. Therefore, minor allergens are those that cause allergy in less than 50% of sensitive individuals (Weerd *et al*., 2002). Allergens are named according to international convention, since in most cases, their natural biochemical function has seldom been established. The convention is that the allergen is named according to the name of the source genus (first three letters) and species (first letter) followed by a number, for example, the allergens of rye-grass, *Lolium perenne,* are named Lol p 1, Lol p 2, Lol p 3, etc. (King et al., 1994). Allergens from the tree *Betula verrucosa* are known as 'Bet v' allergens (Weerd *et al*., 2002). Allergens are named and classified according to their level of immunological reactivity (King *et al.,* 1994). Furthermore, these proteins are classified into groups based on similar structural properties and immunological cross-reactivity with other allergens from similar sources (Weerd *et al*., 2002).

Pollen contains many proteins but only a small portion of these has the ability to interact with the human immune system to cause allergic symptoms. Certain biochemical properties of allergenic proteins contribute to this interaction, including localization of allergens within pollen, rapidity of solubilization upon pollen hydration and recognition of components of the proteins by the human immune system (Knox & Heslop-Harrison, 1970; Singh *et al*.,1991; Arnon & Van Regenmortel, 1992; Vrtala *et al*., 1993). Genes and cDNA sequences encoding allergenic proteins from the pollen of many grass, tree, weed and crop species have subsequently been cloned and sequenced. Cloning and recombinant expression of pollen allergens has allowed determination of molecular and immunological characteristics of these proteins including identification of the sites that interact with the human immune system and therefore lead to the development of allergy symptoms. Structural similarities with functional proteins and enzymes have been identified for a number of pollen allergens, suggesting clues as to the function of these proteins. Cloning of pollen allergens has also allowed identification of immunologically cross-reactive proteins in pollen from taxonomically related and distant plant species, and plant-derived food extracts. These observations have helped to explain the incidence of sensitivity to pollen and foods derived from distantly related plant species often displayed by pollen allergic patients (Weerd *et al*., 2002).

Aeroallergens are present as a natural part of the atmosphere, where they occur in the form of dust, aerosols, cellular material, particles as small as 0.01 µm, and can be deposited on the mucous membranes of the eyes, nasal and oral cavity and airways. Aeroallergens include reproductive structures of many seed plants (pollen grains) and fungi (spores), as well as certain algae, bacteria, actinomycetes and protozoa, which regularly undergo atmospheric transportation. In addition, biogenic debris, including mammalian danders and arthropod emanations, are also aeroallergens (Solomon & Mathews, 1988). Aeroallergens of plants can be derived from pollen of conifers and flowering plants, seeds, leaf and stem detritus and protein molecules, especially proteolytic enzymes. These allergens are a natural component of the atmosphere either through their biological activity or human activity (Ong *et al.,* 1995).

The spectrum of air particles causing allergic rhinitis and asthma is rather well known. There are regional differences both in occurrence and allergenicity of pollen and spores. Due to human activities, we must await slight floral changes within a decade. Therefore, changes of the allergen content of the air should be observed, to react early enough with changes in diagnostic and therapy means whenever a new allergen should appear. If one or the other well known aeroallergen would show a significant trend to disappear, the obvious things will be easily done, but new allergens demand some time for organization of tests and therapeutical solutions (Jager, 1989).

Symptoms of sensitivity to allergens in pollen typically develop upon contact of pollen with the mucosal surfaces of the eyes, nose and throat of sensitized individuals (Bush, 1989). This typically results in the development of conjunctivitis, nasal congestion, sneezing and irritation of the eyes, nose and throat. Hay fever is often the result of inhalation of whole pollen or large pollen particles (Wilson *et al.,* 1973). Smaller pollen particles and allergen-carrying components can often be inhaled into the lower airways where they induce the development of asthmatic symptoms in sensitive individuals, including breathlessness, mucosal inflammation and hypersecretion, muscle hyper-reactivity and anxiety (Stewart & Holt, 1985).

Pollinosis is the most common allergic disorder. As is well known, the clinical manifestations of pollinosis are determined by a specific hypersensitivity to pollen, especially anemophilous pollens of certain plants, among genetically susceptible individuals (Pacini, 1990). A person can only develop pollinosis when he is exposed to pollen with allergenic properties. The severity of the symptoms depends in part on the frequency of exposures and on the amount of pollen per exposure. Most exposures will be to pollen present in the air in relatively high concentrations **(**Driessen & Derksen, 1989). In practice this means that pollinosis is mostly caused by plants that depend on the wind for cross-fertilization: the wind-pollinating species. Occurence in the air depends, apart from meteorological variables, on the weight/surface ratio of the pollen: small, light pollen will more readily be carried by wind than relatively large and heavy pollen. The texture of the surface might also be relevant to dissemination by the wind. However, differentiation in texture is a far from constant feature of windpollination species; it is probably of only little importance for dissemination by wind **(**Driessen & Derksen, 1989).

Pollen is indispensable to the reproduction of seed-bearing plants (Spermatophyta) in that it represents the male gametophyte, whose task is to fertilize the omospecific ovules. It should be noted, by the way, that a complex genetic code exists also among vegetables. According to this code the protein antigens of pollen grains contain special markers by which they are recognized and accepted only by species-specific receptors on the pistils, thus preventing any incompatible fecundation (Pacini, 1990).

Not all pollens cause allergy. Not all pollens are equally allergenic. We may regard as strictly allergenic those species of plants which trigger disturbances in a significant number of subjects over large areas (Spieksma, 1990). This means about twenty plants (Negrini, 1992). On this subjects, it is worth remembering the postulates so clearly defined by Thommen in 1931 whereby a pollen may be able to sensitize an atopic subject and thus bring on clinical symptoms (Coca *et al.*,1931):

- it must contain an antigenic component capable of inducing sensitivity;
- it must belong to an anemophilous plant;
- it must be produced in large quantities:
- it must be light enough to be carried long distances:
- it must belong to a plant which grows widely.

Such conditions are undoubtedly fulfilled in tile vast majority of cases. Under exceptional conditions, pollens from entomophilous plants (some Asteraceae) can also give rise to sensitization, for instance as a result of high dispersion in air or direct handling of the plant in flower (Spieksma, 1990). The first of Thommen's postulates listed above is particularly important: the pollen must contain a specific component which causes sensitization (antigen) and which is specifically able to induce an IgE-mediated antibody response (allergen) it can then bind to such antibodies. (Negrini, 1992). In 1971, Wodehouse defined criteria for an allergenic plant. To be allergenic its mode of pollination must be entirely anemophilous and its pollen must be buoyant, abundant and allergenic. This may explain the relatively low incidence of pollinosis caused by anemophilous plants, in relation to the large number of such plants. A clear example is provided by Pinaceae, *Fagus, Quercus* spp. and others. Although in some localities these plants are widely found, they do not very often determine an allergic sensitization; this can probably be explained by the low level of allergenicity of their pollens (Spieksma, 1990).

Humankind co-exists in the global environment with plants. Plants produce propagules, pollen, seeds and chemicals, some of which are a natural part of the atmosphere. Humans come in contact with or inhale these atmospheric particles, which are part of the air they breathe. These particles may carry plant-derived proteins, glycoproteins or polysaccharides, which may act as antigens in interacting with the human immune system. Of special interest are those antigens which act as allergens in eliciting the immediate hypersensitivity response (Ong *et al.,* 1995). This response is mediated by the special atopic antibody, Immunoglobulin E (Druce, 1993). Exposure to allergens leads to: (1) production of specific IgE in the immune system; (2) circulation and binding of IgE to the surface of most cells and basophils; (3) binding of allergen molecules to adjacent pairs of IgE molecules; and (4) release of mediators of the allergic response (Roitt *et al*., 1985). Atopy, defined as the ability to produce IgE antibodies to common allergens, demonstrable by skin prick test reactivity, is clinically manifested as allergic diseases such as asthma, rhinitis, eczema and food and animal sensitivities. With up to 25% of the populations in industrialized countries suffering these diseases, the impact of allergy on society is significant. Of the diseases, allergic rhinitis (known as hay fever or pollinosis) has the highest incidence with 10–20% of the population suffering from this disorder; 5–10% exhibit asthmatic symptoms while 1–3% experience food allergies (Durham & Church, 2001). Whilst food allergy and eczema are often predominant in infancy, asthma and rhinitis often may not develop until late in childhood or early adulthood. Pollen allergy is also typically a seasonal disease often dependent upon the flowering season of the plant to which an individual is pollen-sensitive. However, polysensitized, pollenallergic individuals may suffer symptoms of hay fever almost year-round (Weerd *et al*., 2002).

In addition to various chemical polluting agents the air also carries biological materials which can be health threatening. This is the case for many pollen types from grasses, trees or weeds, which cause symptoms of allergy among the sensitized populations. Determining the amount of each pollen type in the air is a mean for evaluating such a risk. Pollen counts are obtained by sampling air from the atmosphere with different pollen traps and then by identifying and counting the pollen types of interest (Cour, 1974). Pollen counts are useful in at least two instances. In the short run, pollen counts help patients and physicians to adapt the medication to the level of the offending pollen in the air. In the long-term, pollen counts are the most convenient way to evaluate the spreading or the decrease of the plant in question (Dechamp *et al*., 1997).

MATERIAL AND METHODS

The pollen aspiration was made by means of a volumetric collector type VPPS 2000 Lanzoni, situated on the buildings of the West University of Timişoara, at a height of approximately 20 m (Faur *et al*., 2003; Ianovici & Faur, 2001). The qualitative and quantitative analysis of pollen grains in the aeroplankton was performed according to the IAA regulations. Analysis of the allergen pollen count and pollen fall distribution was performed on the basis of the data collected in Timişoara in the seasons of 1999–2007. We have drawn the pollen calendar taking into account the results of the monitorisation which took place in 2007 (Ianovici, 2007a; Mandrioli *et al*.,1998).

RESULTS AND DISCUSSIONS

Allergy to the pollen of flowering plant species significantly impacts on the health of people in many parts of the world. With up to 20% of the populations in industrialized countries suffering from pollen allergy, much research has been conducted to identify allergenic pollen species and the allergy-causing components present in their pollen (Weerd *et al*., 2002).

Modern methods of knowing the impact of environment factors, including allergens, hasten to the allergenicity, and the sick people's assistance. One of these methods, extremely valuable in the medical practice, is the volumetric method of sampling, identifying and quantifying the airborne pollen, which belongs to the category of inhaling allergens and which brings about the pollinosis. In the conditions of Timişoara the most important cause of pollinosis is allergenic pollen of some deciduous trees as well as grasses and weeds (Ianovici & Faur, 2001; Ianovici *et al.*, 2003; Ianovici & Faur, 2004; Ianovici & Faur, 2005; Ianovici, 2007a). The vegetation is in the region typical urban because of the introduction of ornamental plants and trees.

Pollen grains of allergenic taxa occur in the atmosphere of Timişoara in large quantities from early February untill late October. It is clear that the Romanian pollen seasons show 3 main parts: tree season (February–April), grass season (May–July), weed season (July–October) (Ianovici, 2007b).

TABLE 1. Vernal pollen season (after Spieksma, 1990)

Family name	Genus name	Commonest species	Allergenic importance
Corylaceae	Corylus	*Corylus avellana*	●●
	Carpinus	*Carpinus betulus*	?
Betulaceae	Betula	*Betula pendula*	●●●
	Alnus	*Alnus glutinosa*	●●
Fagaceae	Quercus	*Quercus robur*	●
Ulmaceae	Ulmus	*Ulmus laevis*	●
Salicaceae	Populus	*Populus alba*	?
	Salix	*Salix alba*	●
Platanaceae	Platanus	*Platanus hybrida*	?
Oleaceae	Fraxinus	*Fraxinus excelsior*	?
Taxaceae/Cupressaceae	Taxus	*Taxus baccata*	?
	Juniperus	*Juniperus commnunis*	●
●●●=major;●●=moderate;●=minor;?=little or unknown			

TABLE 2. Early summer pollen season (after Spieksma, 1990)

Family name	Genus name	Commonest species	Allergenic importance
Poaceae	**a) Wild grasses**		
	Agropyron	*Agropyron repens*	●●
	Agrostis	*Agrostis stolonifera*	●●
	Alopecurus	*Alopecurus pratensis*	●●
	Bromus	*Bromus sterilis*	●●
	Festuca	*Festuca pratensis*	●●
	Lolium	*Lolium perenne*	●●●
	Phleum	*Phleum pratense*	●●●
	Poa	*Poa angustifolia*	●●●
	Cynodon	*Cynodon dactylon*	●●●
	Dactylis	*Dactylis glomerata*	●●●
	Setaria	*Setaria glauca*	●
	Echinochloa	*Echinochloa crus-galli*	●●
	Tragus	*Tragus communis*	●
	Phragmites	*Phragmites australis*	●
	b) Cerealia		
	Secale	*Secale cereale*	●●●
	Zea	*Zea mays*	●
●●●=major;●●=moderate;●=minor;?=little or unknown			

TABLE 3. Late summer pollen season (after Spieksma, 1990)

Family name	Genus name	Commonest species	Allergenic importance
Asteraceae	Ambrosia	*Ambrosia artemisiifolia*	●●●
	Artemisia	*Artemisia vulgaris*	●●●
Chenopodiaceae/Amaranthaceae	Amaranthus	*Amaranthus retroflexus*	●
	Chenopodium	*Chenopodium patula*	●
Urticaceae	Urtica	*Urtica dioica*	?
Plantaginaceae	Plantago	*Plantago lanceolata*	●
Polygonaceae	Rumex	*Rumex acetosella*	●
●●●=major;●●=moderate;●=minor;?=little or unknown			

Monthly variations of total pollen grains recorded in the atmosphere of Timişoara during 2007 are shown in figure 1. The pollen calendar was constructed for *Alnus, Betula, Corylus, Carpinus, Fraxinus, Platanus, Populus, Salix, Ulmus, Taxaceae/Cupressaceae, Quercus, Poaceae, Ambrosia, Artemisia, Chenopodiaceae/Amaranthaceae, Plantago, Rumex, Urtica* taxa producing the allergenic pollen.

Airborne pollen of allergenic plants was found to predominate in the air of the city of Timisoara. Classification of the plant species into groups of trees, grasses and weeds reveals exclusively tree airborne pollen to be found in March and April then in May and June the grass and weed pollen occurred, whereas an absolute predominance of weed pollen was recorded in July, August and September. Airpalynologic values recorded during the years 1999- 2007 show huge differences for our geographic area concerning the incidence of the pollen of some plants with airborne allergen pollen. For instance, we notice the abundance of the pollen coming from a species which is adventives in our country's flora: the *Ambrosia artemisiifolia.* This leads to the necessity of individual testing with *Ambrosia* pollen and the complete study of this plant, in expansion in our country (Faur *et al*., 2003; Ianovici, 2007c)**.**

The mode of environmental transmission of pollen can influence the level and incidence of exposure to allergens by sensitized individuals, with wind pollinated plant species posing a greater risk of exposure than insect pollinated species (Smart et al., 1979; Solomon, 1984; Jimenez *et al*.,1994). Furthermore, the interaction between aeroallergens and particulate and gaseous air pollutants, and the presence of allergen-containing, paucimicronic particles in the atmosphere have also recently been shown to be associated with allergic sensitization (Behrendt *et al.,* 1997; Behrendt *et al*., 1995). Pollen allergy has a remarkable clinical impact all over Europe, and there is a body of evidence suggesting that the prevalence of respiratory allergic reactions induced by pollens in Europe has been on the increase in the past decades (D'Amato, 2001; D'Amato *et al.,* 1992; D'Amato *et al.,* 1998; ECRHS, 1996; ISAAC, 1998; Burney *et al.,* 1997).

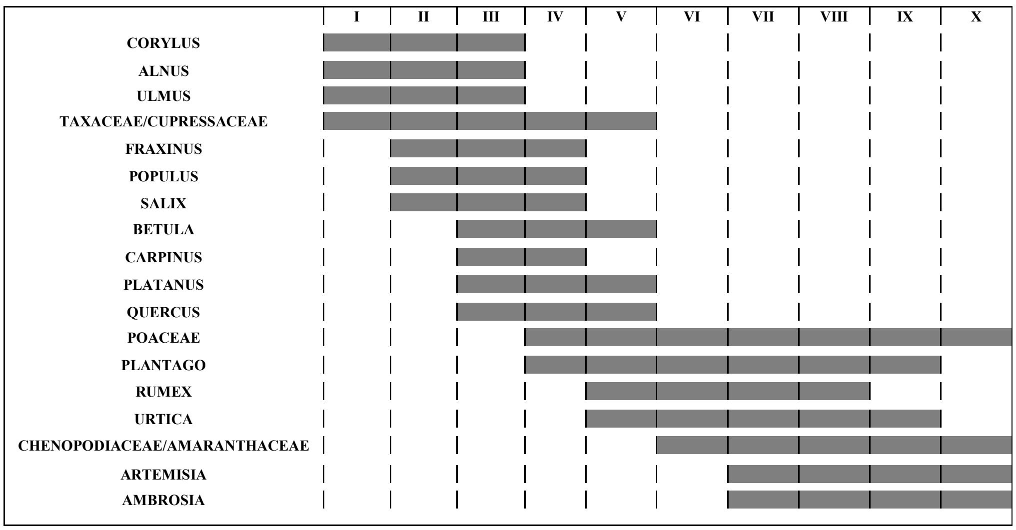

FIG.1. Allergenic pollen calendar of 2007

However, recent findings of the phase three of the International Study of Asthma and Allergies in Children (ISAAC) study showed the absence of increases or little changes in prevalence of asthma symptoms, allergic rhinoconjunctivitis and eczema for European centres with the existing high prevalence among the older children (Asher *et al.,* 2006). The prevalence of pollen allergy is presently estimated to be up 40%. Exposure to allergens represents a key factor among the environmental determinants of asthma, which include air pollution (Eder *et al*., 2006). Since airborne-induced respiratory allergy does not recognize national frontiers, the study of pollinosis cannot be limited to national boundaries, as obviously happens with most diseases that can be prevented by avoiding exposure to the causative agent. In Europe, the main pollination period covers about half the year, from spring to autumn, and the distribution of airborne pollen taxa of allergological interest is related to five vegetational areas (D'Amato *et al.,* 2007).

TABLE 4. Vegetational areas and prevalent distribution of allergenic plants in Europe

Arctic: birch
Central: deciduous forest, birch, grasses
Eastern: grasses, mugwort, ragweed
Mountains: grasses (with a pollination season delayed by three-four weeks in comparison with areas at sea level)
Mediterranean: Parietaria, olive trees, grasses and also cypress.

The allergenic content of the atmosphere varies according to climate, geography and vegetation. Data on the presence and prevalence of allergenic airborne pollens, obtained from both aerobiological studies and allergological investigations, make it possible to design pollen calendars with the approximate flowering period of the plants in the sampling area. In this way, even though pollen production and dispersal from year to year depend on the patterns of preseason weather and on the conditions prevailing at the time of anthesis, it is usually possible to forecast the chances of encountering high atmospheric allergenic pollen concentrations in different areas. Aerobiological and allergological studies show that the pollen map of Europe is changing also as a result of cultural factors (for example, importation of plants such as birch and cypress for urban parklands), greater international travel (e.g. colonization by *Ambrosia* in France, northern Italy, Austria, Hungary etc.) and climate change. In this regard, the higher frequency of weather extremes, like thunderstorms, and increasing episodes of long range transport of allergenic pollen represent new challenges for researchers. Furthermore, in the last few years, experimental data on pollen and subpollen-particles structure, the pathogenetic role of pollen and the interaction between pollen and air pollutants, gave new insights into the mechanisms of respiratory allergic diseases (D'Amato *et al.,* 2007).

Pollen is present in the atmosphere almost year round. Whilst allergenic pollen species may account for only 20 to 30% of the total annual pollen catch, they represent the predominant species during the respective flowering seasons. Trees begin flowering in winter and are the major source of airborne pollen allergens in northern Europe (D'Amato *et al*., 1998). Grasses flower through spring and summer and are the major source of airborne pollen allergens in Australia, California, many parts of Europe and other cooltemperate climates (Smart *et al.,* 1979; Spieksma,1990; D'Amato *et al*., 1998). Weeds, such as those of the *Ambrosia* (ragweed) and *Artemisia* (mugwort) genera, flower in late summer and are the predominant source of pollen allergens in North America and eastern Europe (King, 1976; D'Amato *et al*., 1998).

The prevalence of pollen-related allergies, asthma and rhinitis, has incresased worldwide. Several causes of this phenomenon have been suggested, such as air pollution, infections and other environmental factors. Without doubt, the amount of allergens, and possibly their allergenicity, has increased and therefore the number of allergic reactions may also be enhanced. Allergic symptoms are related to the composition, timing and abundance of airborne allergenic material, together with other environmental and genetic risk factors. A definition of the concept of "allergenic plant" is hard to frame. Most allergenic plants are anemophylous and disperse pollens by air currents. Hovewer, in small groups of people under local conditions zoophilous plants, which pollens are transported by animals, (more commonly insects), may be responsible for pollinosis symptoms. Criteria for not allergenic plants selection was (Lorenzoni-Chiesura *et al*., 2000):

- enthomophylus or zoophylus plants;
- no evidence of specific sensitization as reported in the literature, or only anedoctical case-reports about few cases of allergy.

Many of the plants cultivated in urban areas turn out to be responsible – alone or in association with other plants or other allergens (acaruses, animal byproducts, fungi, foods, etc.) – for pollinosis or mixed allergies and their importance in this field seems to be constantly increasing. It certainly does not seem logical to eliminate, strong and well-settled trees located in city areas, it would be desirable and important instead that the town councillorships in charge of public parks and garden, farmers, breeders, gardeners, always keep in mind also these characteristics (other than shape, colour of foliage, morphology of leaves, flowers, fruits, etc.) of the species they plant, in order to avoid a problem that causes suffering to many people, and the loss of many working hours. The most important species which should be avoided now are mostly those causing sensitizations, indicated as springtime allergies (*Alnus* sp., *Carpinus* sp., *Corylus avellana*, *Cryptomeria japonica*, *Cupressus* and *Fraxinus* sp.), on the other hand, other species should not be overlooked. It is still unknown why a species, considered for a long time of low allergenicity can suddenly increase its importance (as it happened, for example, with *Corylus avellana*). Not-allergenic plants may be both autochtonal and allochtonal. Some exotic plants are also recommended in order to avoid sensitization (Lorenzoni-Chiesura *et al*., 2000).

TABLE 5. Summarize the list of recommended plants in Timişoara

FAMILY	SPECIES
Berberidaceae	*Berberis julianae* C.K. Schneider
	Mahonia acquifolium (Pursh) Nutt.
Bignoniaceae	*Tecoma radicans* (L.) Juss.
Caprifoliaceae	*Viburnum opulus* L.
	Viburnum rhytidophyllum Hemsl. Ex Forb & Hemsl.
Cornaceae	*Cornus mas* L.
	Cornus sanguinea L.
Ginkgoaceae	*Ginkgo biloba* L.
Lamiaceae	*Lavandula angustifolia* Miller
	Rosmarinus officinalis L.
Fabaceae	*Ceratonia siliqua* L.
	Cercis siliquastrum L.
	Gleditsia triacanthos L.
	Sophora japonica L.
Magnoliaceae	*Liriodendron tulipifera* L.
	Magnolia grandiflora L.
	Magnolia soulangiana Hort (ex Thieb.)
	Magnolia stellata Maxim
Malvaceae	*Hibiscus syriacus* L.
Moraceae	*Ficus carica* L.
	Maclura pomifera (Rafin.) C.K. Schneider
Oleaceae	*Forsythia viridissima* Lindl.
Rosaceae	*Crataegus monogyna* Jacq
	Malus domestica Borkh
	Mespilus germanica L.
	Prunus laurocerasus L.
	Pyrus communis L.
	Spiraea vanhuttei Zabel
Scrophulariaceae	*Paulownia tomentosa* (Sprengel) Steudel
Tamaricaceae	*Tamarix gallica* L.

The adaptation of foreign plants to new climate conditions and environment is not easily predictable. In particular local condition, in which high pollen concentration can be reached, the possibility of sensitization in small groups of peoples must be kept in mind (Lorenzoni-Chiesura *et al*., 2000).

CONCLUSIONS

Allergenic pollen taxa identified in Timişoara during monitoring throughout 1999-2007 was: *Alnus, Betula, Corylus, Carpinus, Fraxinus, Platanus, Populus, Salix, Ulmus, Taxaceae/Cupressaceae, Quercus, Poaceae, Ambrosia, Artemisia, Chenopodiaceae/Amaranthaceae, Plantago, Rumex, Urtica*. More attention must be paid to these taxa, especially in managing of allergy problems in urban areas. *Ambrosia artemisiifolia*, the most allergenic plant of our climate, was in full bloom in August.

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