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AIRBORNE RAGWEED (*AMBROSIA ARTEMISIIFOLIA* L.) POLLEN CONTENT IN THE CITY OF ZAGREB AND IMPLICATIONS ON POLLEN ALLERGY

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Abstract: The increasing problem of sensitization to *Ambrosia* pollen in Europe has stimulated studies on *Ambrosia* pollen occurrence and pollinosis in Croatia. The aim of this study was to analyze the ragweed pollen pattern in Zagreb and to determine the incidence of sensitization to this pollen types in patients with seasonal respiratory allergy. The study was performed in the city of Zagreb during the 2002-2005 seasons. Skin prick test were performed on a total of 750 patients aged 18–80 in Zagreb between 2 January – 31 December 2004. The mean 4-year rate of ragweed pollen in the Zagreb atmosphere was 14.8% of all plant pollen. The highest rate of airborne ragweed pollen was recorded in August and September. 365 patients were allergic to ragweed pollen; 20.3% of them monosensitized; 10.9% allergic to *Ambrosia* and *Artemisia* pollen. Almost all study patients suffered the most severe symptoms in August and September. The highest proportion of allergic patients were recorded in the 31-50 age group and the lowest proportion in the >50 age group. Forecasting of allergenic pollen season in an area is a crucial pursuit for all developed countries, in order to minimize clinical symptoms in patients suffering from respiratory allergy. This can be achieved through public announcements by mass media (radio, television, internet, etc.) aiming to protect allergic individuals.

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INTRODUCTION

Pollen allergy (pollinosis) is a common disease caused by hypersensitivity reaction of the respiratory tract and eye conjunctivae to pollen grains. It refers to seasonal allergic manifestations affecting patients during the plant pollen season. The characteristic of seasonal allergy is the recurrence of symptoms. It is well established that inhalation of pollen grains induces respiratory allergy symptoms in sensitized individuals. These are clinically manifested as rhinitis, rhinoconjunctivitis and bronchial asthma [12]. Aerobiological and clinical studies from various cities in the USA have documented the importance of ragweed pollen as an aeroallergen [10]. In Europe, the severity of ragweed pollinosis varies according to geographical region. Positive results of skin prick test or positive RAST reactions to ragweed allergens in pollen allergic patients reached the following values: Hungary, more than 80% [25], northern Italy, nearly 70% [7, 33] France, 30% [5], Czech Republic, about 35% [35], Austria, about 30% [18], and southern Switzerland, 17% [11]. The high frequency of sensitization might be caused by the apparent high degree of cross-reactivity with various other members of the family Asteraceae, and the families Poaceae and Betulaceae [13, 21, 31, 32]. Dose response relationships between pollen concentrations and clinical symptoms are scarce. There is no unique criterion for establishing the minimum level capable of triggering allergic symptomatology since the

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response is highly individual. However, it has been pointed out that ragweed pollen concentrations greater than 20–30 grains in m^3 per 24 h trigger severe symptoms [17, 24, 40]. The increasing problem of sensitization to Ambrosia pollen in Europe has stimulated studies on Ambrosia pollen occurrence and pollinosis in Croatia.

The aim of this study was to analyze the ragweed pollen pattern in Zagreb (2002–2005) and to determine the incidence of sensitization to this pollen type in patients with seasonal respiratory allergy.

MATERIAL AND METHODS

The study was performed in the city of Zagreb during the 2002-2005 seasons. A 7-day VPPS 2000 Hirst volumetric spore trap (Lanzoni, Bologna, Italy) was used for pollen sampling. The sampler was placed at 19.7 m on the roof of the Grič Observatory in the centre of the city of Zagreb (45°49’ N and 15°59’ E, 157 m above the sea level). The tape was removed twice weekly, cut to a length corresponding to 24-h pollen sampling, applied onto a glass slide and embedded [27]. Samples were examined under a light microscope, magnification ×400, to determine pollen type and count per 1 m^3 air. Five horizontal sweeps on each slide were analyzed. The minimum number of horizontal sweeps is the one that assures a total observed area which is at least 20% of the sampled area. The accuracy of the measurement is proportional to the number of sweeps and the concentration of particles [8]. Pollen concentration was expressed as pollen grain count/m^3 air.

For this study, a total of 750 patients aged 18–50< were examined (history and skin-prick test) who presented for the first time to Jordanovac University Hospital for Lung Diseases and Zagreb Polyclinic for Lung Diseases between 2 January and 31 December 2004. Skin prick tests were performed by the usual method. Allergen preparations of grass pollen – *Alopecurus pratensis*, *Agrostis alba*, *Anthoxantum odoratum*, *Cynodon dactylon*, *Dactylis glomerata*, *Festuca elatior*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Secale cereale*, *Triticum sativa*, *Zea mays*; weed pollen – *Ambrosia elatior*, *Artemisia vulgaris*, *Artemisia absinthium*, *Solidago canadensis*, *Rumex acetosella*, *Plantago lanceolata*, *Parietaria judaica*, *Parietaria officinalis*, *Taraxacum officinale*, *Urtica dioica*, *Humulus lupulus*, *Cannabis sativa*; and tree pollen – *Corylus avellana*, *Betula verrucosa*, *Sambucus nigra*, *Tilia cordata*, *Pinus nigra*, *Robinia pseudoacacia*, *Salix alba*, *Populus alba*, *Platanus acerifolia*, *Alnus incana*, *Quercus robur*, *Aesculus hippocastanum* (Institute of Immunology, Zagreb) were dialyzed, and the extracts dissolved in a solvent, a mixture of 50% glycerol solution in buffered saline with the addition of a stabilizer. A solution of histamine HCl at a concentration of 1 mg/mL (5.43 mmol/l) was used as positive control, and 50% glycerol solution in phosphate buffer as negative control. The mean values of positive reaction had urtica diameter of 5–10 mm, and erythema diameter of 10–30 mm.

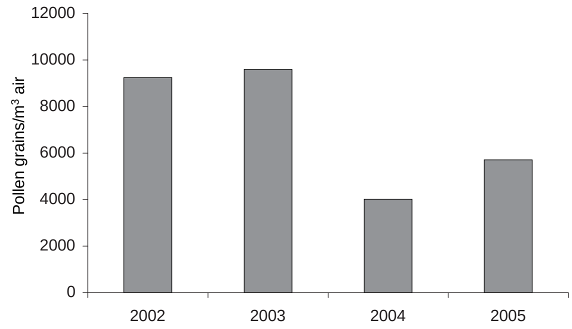

Figure 1. Annual sums of *Ambrosia* airborne pollen in Zagreb 2002-2005.

RESULTS

Analysis of the annual pattern of ragweed pollen concentration revealed it to decrease in 2004 (4,015 p.g./m^3) and 2005 (5,711 p.g./m^3) relative to 2002 (9,243 p.g./m^3) and 2003 (9,601 p.g./m^3) (Fig. 1). The first occurrence of ragweed pollen in the air was recorded from 28 June – 1 August, depending on the year with peak concentration in the second half of August through the first week of September. Throughout the study period, the pollen season terminated in October. The number of days with airborne pollen concentration exceeding the level triggering allergic symptoms (>30 p.g./m^3) was 31–46, also depending on the year (Tab. 1). The mean 4-year rate of ragweed pollen in the Zagreb atmosphere was 14.8% of all plant pollen types. The highest rate of airborne ragweed pollen was recorded in August and September, accounting for 53.1% (August 2002) to 95.5% (September 2003) of all plant pollen types (Tab. 2). Of 750 study patients, 365 were allergic to ragweed

Table 1. Presence of *Ambrosia* pollen in Zagreb atmosphere 2002–2005.

Year	Period of occurrence	Peak day	Conc. on peak day (p/m^3)	No. of days with >30 p/m^3
2002	1/8–5/10	03 September	652	34
2003	28/6–29/10	06 September	883	46
2004	27/7–20/10	19 August	298	31
2005	25/7–30/10	31 August	494	31

Table 2. Monthly pattern of *Ambrosia* airborne pollen in Zagreb, 2002–2005.

Year	Ragweed pollen count/% of ragweed pollen in monthly total pollen count				
	Pollen season				
	Jun	Jul	Aug	Sep	Oct
2002	0.0	0.0	5,917/53.1	3,318/79.9	8/85.4
2003	5/0.1	80/2.9	5,352/70.7	4,131/95.5	33/82.1
2004	0.0	6/0.3	2,190/58.6	1,793/85.1	26/65.7
2005	0.0	50/2.3	2,688/62.0	2,866/89.3	107/69.0

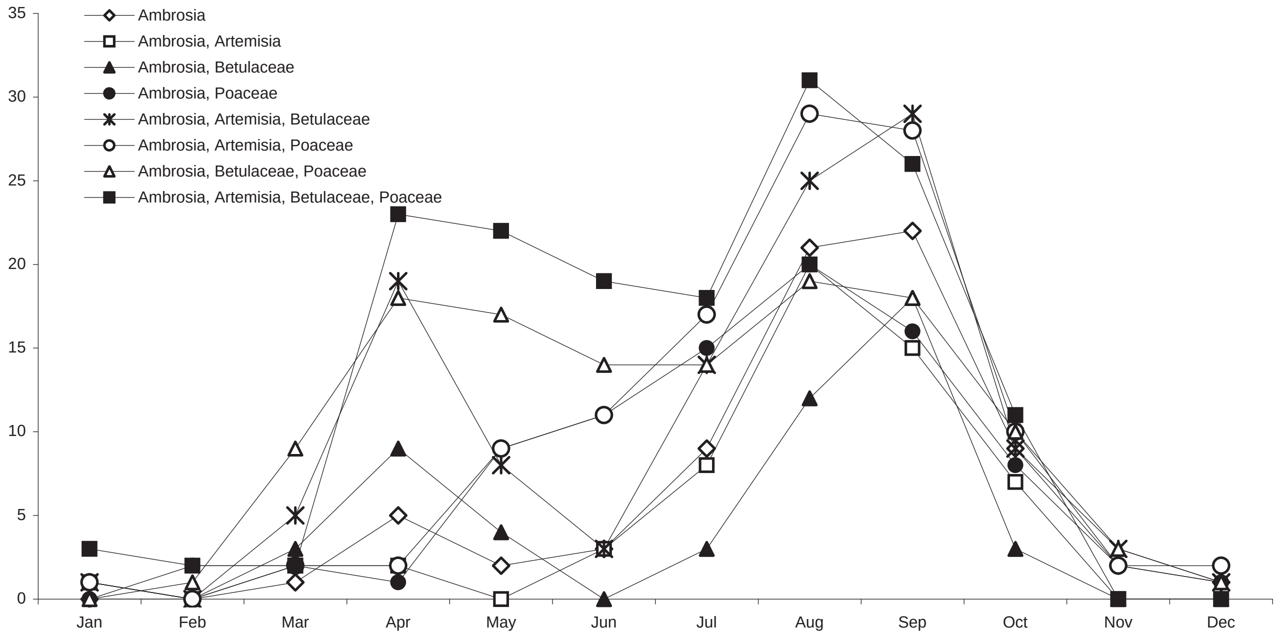

Figure 2. Annual pattern of allergic symptomatology in monosensitized and polysensitized patients.

pollen, 20.3% of them monosensitized, 10.9% allergic to *Ambrosia* and *Artemisia* pollen, whereas the rest were polysensitized to ragweed and mugwort pollen, grass pollen, and pollen of the family Betulaceae. The highest proportion of polysensitized patients (16.9%) were allergic to the *Ambrosia* and Poaceae pollen combination, and the lowest proportion (4.9%) to the *Ambrosia*, *Artemisia* and Betulaceae pollen combination (Tab. 3). Following the airborne pollen distribution depicted above, almost all study patients suffered most severe symptoms in August and September, then in April. In patients monosensitized to ragweed pollen the symptoms were most pronounced in August and September. In patients polysensitized to *Ambrosia* and Betulaceae pollen who developed cross-reaction, the rather pronounced symptoms occurred as early as March, with a minor peak in April and abrupt exacerbation in August and September. Patients allergic to pollen of *Ambrosia*, *Artemisia*, Betulaceae and Poaceae showed the poorest condition, suffering allergy symptoms throughout the year, with exacerbations in spring and late summer (Fig. 2). Sex analysis showed a slight female predominance of allergic individuals (50.69% vs 49.31%), whereas analysis according to age groups yielded a different pattern. Male predominance was recorded in the 18–30 age group

Table 3. Age and sex distribution of respiratory allergy patients according to different pollen type

Sensitized to pollen	Patients (Skin Prick Test Positive) age								Total %
	18-30 (mean age 24)		31-50 (mean age 38)		51< (mean age 58)		Total		
	♂	♀	♂	♀	♂	♀	♂	♀	
Ambrosia	11	6	13	29	4	11	28	46	20.3
Ambrosia, *Artemisia*	2	6	12	8	8	4	22	18	10.9
Ambrosia, Betulaceae	6	5	12	11	6	7	24	23	12.9
Ambrosia, Poaceae	22	13	8	11	5	3	35	27	16.9
Ambrosia, *Artemisia*, Betulaceae	0	1	5	8	2	2	7	11	4.9
Ambrosia, *Artemisia*, Poaceae	7	7	5	10	3	5	15	22	10.2
Ambrosia, Betulaceae, Poaceae	18	10	8	9	2	1	28	20	13.2
Ambrosia, *Artemisia*, Betulaceae, Poaceae	10	6	8	8	3	4	21	18	10.7
Total	76	54	71	94	33	37	180	185	
% (age)	*35.62*		*45.2*		*19.18*		*49.31*	*50.69*	

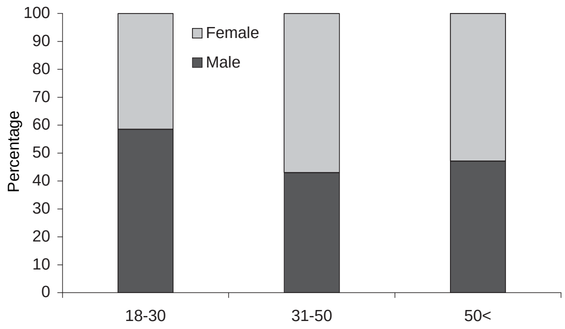

Figure 3. Sex distribution of patients with positive skin-prick test within age groups.

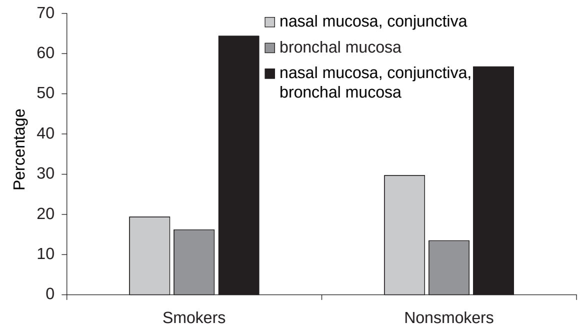

Figure 4. Allergy symptoms according to localization in smokers and nonsmokers.

(58.5%), whereas female allergic individuals prevailed in other age groups (57% and 52.9% in the 31–50 and >50 age group, respectively). The highest proportion of allergic patients were recorded in the 31–50 age group (even 45.2%) and the lowest proportion in the >50 age group (19.8%) (Tab. 3, Fig. 3). Analysis of the allergy symptom manifestations according to airway and mucosal localization in smokers and nonsmokers, allergic mucositis (nasal, bronchial) and conjunctivitis predominated in both groups (smokers 64.4% and nonsmokers 56.8%). Nasal mucosa was more frequently involved in nonsmokers (30%), and bronchial mucosa in smokers (Fig. 4).

DISCUSSION

Pollen allergy is related to local vegetation and depends on the way and duration of exposure to aeroallergens, provided that there is a genetic predisposition to respiratory allergy [14, 26]. Many researchers relate the occurrence of respiratory allergy symptoms to the presence of pollen grains in ambient air; in addition, it is well documented that inhalation of specific types of pollen grains causes clinical symptoms of respiratory allergy. Concentrations of ragweed airborne pollen in the atmosphere of Zagreb vary annually [28, 29]. The mean annual index of ragweed pollen in 2002 and 2003 was 9,422, accounting for 18% of total annual index of all plant pollen, whereas in 2004 and 2005 it fell to 14%. However, these values greatly exceed the values recorded in Geneva (Switzerland) [39] and Szczecin (Poland) [34] for the same period. However, our proportions are still lower than the proportion of ragweed pollen in Montreal (about 30% of total annual pollen count) [3]. A wide range of factors affect the production, dispersion and transportation of airborne pollen (wind direction and speed, rainfall and relative humidity during the flowering season) [37]. Annual variation in the ragweed pollen concentration in the Zagreb atmosphere could be explained by different weather conditions, especially pronounced in 2003 and 2004 [30]. Many studies suggest that it is necessary to record and correlate the airborne pollen concentrations with the most important meteorological parameter values [2, 23]. Maximum ragweed pollen emissions are restricted to the summer months of August and September, the warmest and driest months of the year [20] which is consistent with the data reported for Austria and Switzerland, as well as for countries in the central part of eastern Europe [4, 19, 22]. Exact information on daily pollen concentrations exceeding 30 pollen grains per m^3 air is of great importance to persons with ragweed pollen allergy, because the accumulation of high pollen amounts in the atmosphere produces the effects of pollinosis in the respiratory tract. Our results show that 31–46 days in the years 2002–2005 were the critical period with daily pollen counts over the allergologic threshold, and considerably exceeded the periods reported for Hungary (25–30 days) and Poland (1–5 days) [16, 38].

In our sample of patients with pollen allergy (Zagreb residents), 49% were allergic to ragweed pollen, which is by some 20% higher than the rate reported in France [5], Austria [18] and the Czech Republic [35] but lower than the rate recorded in Milan [7] and Hungary [25]. While in France the majority of patients were found to be monosensitized to ragweed pollen [6], our results yielded only 20.3% of monosensitized patients, whereas the rest of the patients exhibited polysensitization. Polysensitization was recorded for the pollen types characterized by cross-reaction, primarily *Artemisia vulgaris* pollen [21]. Mugwort and ragweed pollen contain a number of cross-reactive allergens, among them the major mugwort allergen Art v 1. These cross-reactive IgE antibodies can result in clinically significant allergic reactions [13]. Strong cross-reactions have also been observed with grass pollen allergens [9], as confirmed by our results (16.9% of patients allergic to ragweed and grass pollen). Ragweed pollen also appears to be cross-reactive with pollen of the family Betulaceae. In the present study, 12.9% of patients showed allergy to ragweed pollen and pollen shed by representatives of the family Betulaceae. Sex distribution of patients (a slight female predominance of allergy in the 31–50 and >50 age groups) is consistent with the respective data for Spain and Portugal [36] but differs from those for Greece (57% of allergic men); however, our data are in agreement with those for Greece in the 18-30 age group, where there were 58% of allergic men [12]. Age is also an important variable. Most studies have focused on students or military recruits. We included patients belonging to 3 age groups (18–30; 31–50 and >51 years). In our population sample the highest frequency of pollinosis was found in the 31–50 age group, as in the Province of Malaga [41]. A study from the Basque Country reports the highest rate of allergy in the 20–30 age group [1].

According to each individual patient's medical history, they displayed increased respiratory symptoms during the peak of pollen circulation season, that is between August and September.

Forecasting of allergenic pollen season in an area is a crucial pursuit for all developed countries, in order to minimize clinical symptoms in patients suffering from respiratory allergy. This can be achieved through public announcements by mass media (radio, television, internet, etc.) aiming to protect allergic individuals. Thus, the total cost of disease treatment is finally reduced; in fact, it is estimated that this mode of massive protective treatment can decrease the number and frequency of visits to medical doctors by 50% [15].

All data are presented in our area for the first time and could provide the basis for practical application in various medical, biologic and other scientific fields.

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