Allergic Rhinitis and Asthma in Southern Croatia: Impact of Sensitization to *Ambrosia elatior*

Slavica Cvitanović[1], Ljubo Znaor[2], Božica Kanceljak-Macan[3], Jelena Macan[3], Ivan Gudelj[1], Dragica Grbić[4]

[1]Department of Pulmonary Diseases, Split University Hospital, Split, Croatia
[2]Ophthalmology Department, Split University Hospital, Split, Croatia
[3]Institute for Medical Research and Occupational Health, Zagreb, Croatia
[4]Matros-Trade Ltd., Split, Croatia

> **Correspondence to:**

Božica Kanceljak-Macan
Institute for Medical Research and Occupational Health
P.O. BOX 291,
10001 Zagreb, Croatia
bkancelj@imi.hr

> **Received:** October 19, 2006
> **Accepted:** January 11, 2007

> **Croat Med J. 2007;48:68-75**

Aim To identify pollen types in southern Croatia and investigate the impact of sensitization to *Ambrosia elatior* (*A. elatior*) on symptoms and treatment of patients with seasonal allergic rhinitis and/or asthma.

Methods The study recruited 120 patients from Split-Dalmatian County with seasonal rhinitis and asthma symptoms and positive skin prick test to one or more common inhaled allergens. Patients with positive skin prick test and increased specific IgE to *A. elatior* (n = 56) were included in the follow-up study during the *A. elatior* pollen season. Rhinitis and asthma symptoms were scored and drug treatment recorded using standardized questionnaires. Also, forced vital capacity (FVC), forced expiratory volume in one second (FEV1), peak expiratory flow (PEF), and eosinophil count in peripheral blood were measured. Type and pollen concentration of *A. elatior* in the air over the nine-week pollen season were determined on the glass slides using the gravimetric method. The results were expressed as the proportion of *A. elatior* pollen in the total pollen.

Results Fifty-six of 120 patients (46.7%) were sensitized to *A. elatior*. Its proportion in total pollen peaked to 12% in the first week of September. Forty-one patients who completed the follow-up study showed a significantly higher score of symptoms during this peak period than in the beginning of the pollen season for seasonal allergic rhinitis (median ±interquartile range, 50 ± 11 vs 7 ± 4; $P<0.001$) and for seasonal allergic asthma (12 ± 2 vs 0 ± 0; $P<0.001$).

Conclusion *A. elatior* is an important cause of seasonal allergic rhinitis and asthma and must be included in the routine diagnostic procedures in southern Croatia.

Ambrosia artemisiifolia L. (Ambrosia elatior L., obični limunđik in Croatian) is short ragweed, which belongs to the family of *Asteraceae* (*Compositae*). In 1941, an investigation identified *A. elatior* in northern Croatia (Podravina, Posavina, Slavonija) and along the Adriatic coast: Zadar, Istria, the island of Krk, Neretva River Delta, and in uncultivated areas in the valley of Sinjsko Polje (1). *A. elatior* arrived in Croatia from North America (2), with the port of Rijeka being one of the possible routes of its arrival (1).

A. elatior has meanwhile spread all over Europe, from the eastern and central European countries, particularly Poland (3,4), Hungary, and Czech Republic (3,5), to Switzerland (6), North Italy (7), and the east of France (8). Over the last four years, several studies investigated the distribution of *A. elatior* pollen in northern Croatia, confirming its presence in clinically significant concentrations and a declining tendency from East to West (9,10). Although the prevalence of seasonal allergic rhinitis and asthma by sensitization to *A. elatior* pollen in northern Croatia is on the increase, there have been no epidemiological studies about this issue. There are actions under way to eliminate *A. elatior* from yards, uncultivated fields, and areas by the rivers and creeks in Osijek (11) and Zagreb.

In our earlier studies of pollen allergy in adult inhabitants of southern Croatia, we investigated sensitization to different pollens specific for the Adriatic coast, particularly *Parietaria species* (12-14). Until recently, we did not investigate sensitization to *A. elatior* pollen because it was not expected to be present in this region in clinically relevant concentrations. Therefore, this allergen was not included in the routine panel of inhaled allergens in allergy diagnosis. As a number of patients with seasonal allergic rhinitis and asthma showed negative skin tests with the routine panel of inhaled allergens between late summer and early autumn, we suspected that the panel did not include some relevant allergens.

The aim of this study was to identify the types of airborne pollen of *A. elatior*, monitor its concentrations in the pollen season (mid-August, September, and October), establish the prevalence of sensitization to its pollen in patients with seasonal allergic rhinitis and asthma, and relation between pollen concentrations, respiratory symptoms, and the use of medications in their treatment.

Patients and methods

Study design and patients

This was a cross-sectional study including patients with seasonal allergic rhinitis and/or asthma in the Splitsko-Dalmatinska County in southern Croatia. It was also a prospective study of symptoms in the sensitized patients and medications used in relation to *A. elatior* pollen count in the pollen season.

The study was performed between mid August and mid October 2003 at the Department of Pulmonary Diseases of the University Hospital Split. A sample of 320 outpatients who experienced seasonal symptoms of rhinitis and/or asthma for at least 2 years in a row underwent physical examination, skin prick testing to routine inhaled allergens and *A. elatior*, as well as ventilatory capacity measurement and specific IgE measurements (Figure 1). A positive skin prick test to inhaled allergens was found in 120 out of 320 patients (37.5%). A positive skin prick test to *A. elatior* and increased specific IgE to *A. elatior* was found in 56 (46.6%) out of 120 patients with positive skin prick test. These patients were recruited for a nine-week *A. elatior* pollen season follow-up. Scores of rhinitis and asthma symptoms and medications were determined using standardized questionnaires for rhinitis and asthma just before pollination, and weekly throughout the pollen season. Out of 56 patients, 15 voluntarily quit the study, and the results were evaluated in the remaining 41 pa-

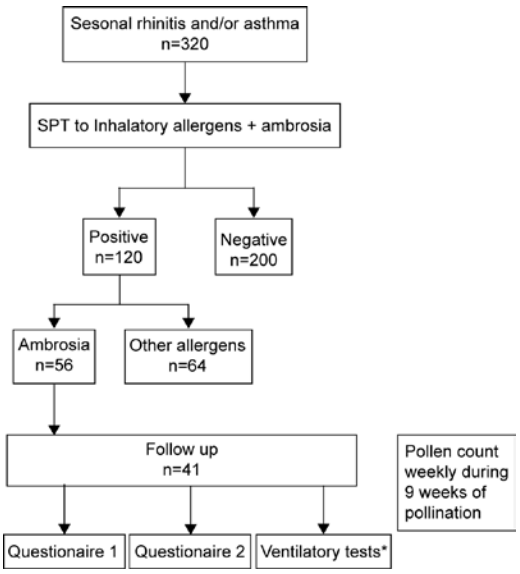

Figure 1. Study design. SPT – skin prick test; questionnaire 1 (15); questionnaire 2 (16); *FVC – forced vital capacity; FEV_1 – forced expiratory volume in 1 second; PEF – peak expiratory flow.

tients. On the last visit, overall assessment of the ambrosia pollen period was made by both the patients and investigators.

Skin Testing

Skin prick tests were performed using a standardized method (17) with inhaled allergens (Institute of Immunology, Zagreb): house dust, *Dermatophagoides pteronyssinus*, hemp, wool, *Olea europea, Parietaria officinalis, A. elatior*, mixtures of tree pollens, weed pollens, molds, feathers, animal dander, and bacteria. All patients were tested with 10 mg/mL histamine hydrochloride and a buffer solution (50% glycerol in phosphate buffer) as control of positive and negative skin reaction, respectively. Skin reaction (wheal) was measured 15-20 minutes after testing. Wheal diameter was calculated according to the formula (D+d/2), where "D" is the longest diameter and "d" is the diameter perpendicular to "D" in mm. Skin reaction was considered positive if the wheal diameter was ≥3 mm greater than the reaction to buffer solution (17,18).

Specific IgE

Specific IgE was determined using the ImmunoCAP technology (UNI CAP 100 – Pharmacia CAP System, Uppsala, Sweden). Values under 0.35 kU_A/L were considered unspecific, whereas values over 0.35 kU_A/L were considered increased (19).

Ventilatory capacity

Ventilatory capacity was measured using the Master Laboratory Spirometer (Jaeger, Würzburg, Germany). Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), and peak expiratory flow (PEF) were determined using a standard method (20). At least three measurements were recorded and the best values were used for analysis.

Eosinophil count in peripheral blood

Eosinophil count in peripheral blood was determined on automatic cell counter CELL-DYN Saphire (Abbott Laboratories GmbH & Co, Wiesbaden-Delkenheim, Germany).

Questionnaire for rhinitis

Nasal symptoms (itching, sneezing, secretion, obstruction) and ocular symptoms (itching, redness, lacrimation) were recorded using a questionnaire adapted from Spector et al (15). Patients recorded and scored symptoms in a diary (0 – no symptoms; 1 – mild symptoms; 2 – moderate symptoms; 3 – severe symptoms). In addition, every used dose of decongestives, antihistaminics, and inhaled corticosteroids was marked as 1.

Questionnaire for asthma

Asthma symptoms were recorded using a questionnaire adapted from Broberger et al (16). Symptoms were noted every day during the whole observation period on the scoring cards. Daytime symptoms score included: 0 – no symptoms; 1 – wheezing; 2 – transitory asthma attacks; 3 – permanent asthma. Overnight symptoms score included: 0 – no symptoms; 1

– wheezing; 2 – 1-2 asthma attacks; 3 – more than 3 asthma attacks. Any additional medication required for the treatment of asthma was also recorded every day. Every dose of short-acting beta-2 agonist or theophylline was marked as 1 and every dose of inhaled corticosteroids was marked as 4.

Pollen count

Pollen concentrations in the air were determined using the gravimetric method (21). Glass slides with a surface of 16 mm^2 were distributed to 4 spots in Split and four spots in Sinjsko Polje. The slides were read every week for 9 weeks from mid-August to mid-October. The results were presented as percentages of *A. elatior* pollen grains in the total pollen grain count on a glass slide (21).

Statistical analysis

Due to small sample sizes and non-normal distributions, data were described using median and interquartile ranges (C±Q). Rhinitis symptom and medication scores, asthma symptom and medication scores, measurements of ventilatory capacity and peripheral blood count for different time points were compared using Friedman test. When Friedman test showed a statistically significant difference, Wilcoxon signed rank test was used for pairwise comparisons of time points at the beginning (first week), the peek (third week), and the end (ninth week) of the pollen season.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS Inc, Chicago IL, USA). Significance level was set at 0.05.

Results

At the beginning, the follow-up study included 56 patients with confirmed sensitization to the *A. elatior* pollen (positive skin prick test, increased specific IgE, and symptoms of seasonal rhinitis and/or asthma) of whom 42 (75%) were monosensitized. This study shows the results of 41 patients who completed the follow-up (Figure 1).

Most patients (28/41, 68.2%) lived in the area around Sinj, whereas others lived in Split and its surroundings (Table 1).

Table 1. Characteristics of patients in the follow-up study

	Patients	
Patient characteristics	male (n = 19)	female (n = 22)
Age (years, C±Q)*	34.8 ± 14.6	30.9 ± 13.5
Family history:		
positive	9	6
negative	10	16
Place of residence:		
Split	2	6
Solin-Klis	2	1
Otok-Sinj	14	14
Brač	1	1

*Median and interquartile range.

All patients from Sinj area reported that *A. elatior* grew in their neighborhood and that their symptoms coincided with its blooming period and started withdrawing as it ended.

All 41 patients reported sneezing, nasal secretion or stuffiness; 10 (24.4%) reported red, itchy, watery eyes, and 9 (21.9%) shortness of breath, coughing, and wheezing. All patients reported that reactions were most intensive outdoors and that they changed with location.

Subjects with positive skin prick test to *A. elatior* pollen allergen had high levels of specific IgE (class 3 and more; median value 45, range 16-100 kU/L).

Fourteen (34.1%) subjects had concomitant hypersensitivity to *Parietaria officinalis* pollen or grass pollens (Figure 2).

A. elatior pollen proportion in total weekly pollen counts over the 9 weeks of pollination season (mid-August to mid-October) started with 4% in the first week, peaked in the third and fourth week with 12% and 10%, respectively, and dropped in the eighth and ninth week to 4% and 3%, respectively (Figure 3).

Seasonal allergic rhinitis symptoms score was significantly higher in the third week of the

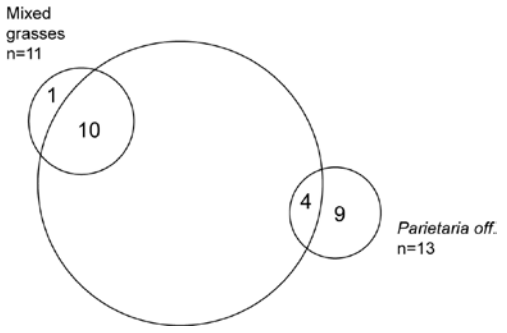

Figure 2. Patients with positive skin prick test to *A. elatior* (n = 56) and to grass pollen and *Parietaria officinalis*.

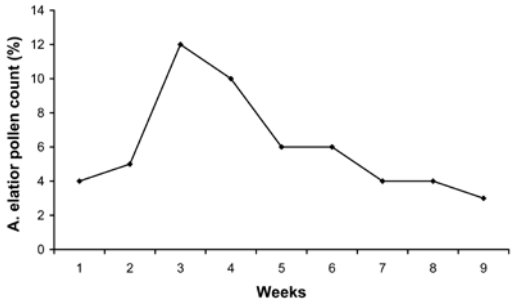

Figure 3. Pollen count during 9 weeks of pollen season.

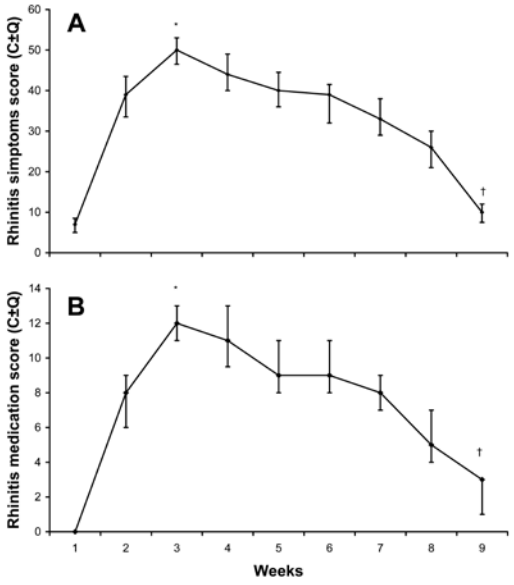

Figure 4. (**A**) Rhinitis symptom scores during 9 weeks of polen season. Asterisk signifies statistically significant difference from weeks 1 and 9 (Wilcoxon signed rank test, *P*<0.001 for both comparisons); Cross signifies statistically significant difference from week 1 (Wilcoxon signed rank test, *P*<0.001); (**B**) Rhinitis medication scores. Asterisk signifies statistically significant difference from weeks 1 and 9 (Wilcoxon signed rank test, *P*<0.001 for both comparisons); cross signifies statistically significant difference from week 1 (Wilcoxon signed rank test, *P*<0.001).

pollen season, when *A .elatior* pollen concentration reached its peak, than at the beginning (first week) and the end (ninth week) of the pollen season (Figure 4A). A significant difference was also found between rhinitis symptoms score in the first and ninth week of the pollen season (Figure 4A).

Seasonal allergic rhinitis therapy score was significantly higher in the third week than in the first and ninth week of the pollen season (Figure 4B). A significant difference was also found between rhinitis therapy score in the first and ninth week (Figure 4B).

Seasonal allergic asthma symptoms score was significantly higher in the third week than in the first and ninth week of the pollen season (Figure 5A). A significant difference was also found between the symptoms score in the first and ninth week (Figure 5A).

Seasonal allergic asthma therapy score was significantly higher in the third week than in the first and ninth week of the pollen season (Figure

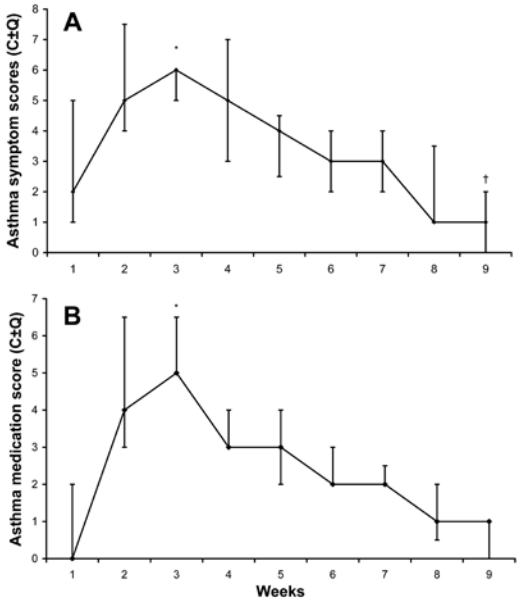

Figure 5. (**A**) Asthma symptom scores during 9 weeks of polen season. Asterisk signifies statistically significant difference from weeks 1 and 9 (Wilcoxon signed rank test, *P*<0.001 for both comparisons); Cross signifies statistically significant difference from week 1 (Wilcoxon signed rank test, *P*<0.001); (**B**) Asthma medication scores. Asterisk signifies statistically significant difference from week 1 (Wilcoxon signed rank test, *P*<0.001).

5B). There was no significant difference between asthma therapy score in the first and ninth week of the pollen season (Figure 5B).

The analysis of ventilatory parameters in patients with seasonal allergic asthma during the pollen season showed a significant differences between the beginning (first week) and peak (third week) of the pollen season (Table 2).

Table 2. Ventilatory capacity measurement and through pollen season in 9 patients with seasonal allergic rhinitis and asthma*

Ventilatory capacity parameters*	Values in pollen season (C±Q)		
	week 1 (beginning)	week 3 (peak)	week 9 (end)
FVC (L)	3.5 ± 0.4†	3.4 ± 0.3	3.4 ± 0
FEV_1 (L)	2.6 ± 0.3‡	2.4 ± 0.3	2.6 ± 0.5
PEF (L/s)	6.3 ± 1.6	5 ± 1.9‖	6.3 ± 1.3

*FVC – forced vital capacity; FEV_1 – forced expiratory volume in one second; PEF – peak expiratory flow; C+Q – median and interquartile range.
† Statistically significant difference from weeks 3 and 9 (Wilcoxon signed rank test, P=0.049 and P=0.013, respectively).
‡ Statistically significant difference from week 3 (Wilcoxon signed rank test, P=0.028).
‖ Statistically significant difference from weeks 1 and 9 (Wilcoxon signed rank test, P=0.008 for both comparisons).

For all patients, eosinophil count in peripheral blood was significantly higher in the third week (C±Q=12±5) than in the first (C±Q=6±3) or ninth (C±Q=6±1) week of the pollen season (Wilcoxon signed rank test, P<0.001 for both comparisons)

Discussion

This study was performed in a group of patients who lived in the southern part of Croatia with the Mediterranean climate. A significant proportion of patients seeking medical help due to allergic respiratory symptoms in the late summer and early autumn (56/120, 47%) were sensitized to *A. elatior*. Sensitization was confirmed in all patients with positive skin prick test and increased levels of specific IgE (UNI-CAP class 3 or more). Most patients included in the study (28/41, 68%) inhabited the surroundings of the town of Sinj, where *A. elatior* was identified some fifty years ago (22). The rest of the patients (13/41, 31.7%) lived in the city of Split and its surroundings. The specificity and significance of *A. elatior* pollen in the etiology of respiratory disorders in this region are underlined by the high proportion of monosensitized patients (42/56, 75%). Results of our study confirm the assumption that airborne *A. elatior* pollen grains are ubiquitous on Adriatic coast too. Furthermore we found cause-and-effect connection between pollen count and clinical symptoms in sensitized subjects.

The *A. elatior* pollen was identified in total pollen count in the period from mid August until mid October, with its peak value in the first week of September. So far, no other species related to *A. elatior* have been found in the uncultivated areas around Sinj, which confirms the specificity of sensitization to *A. elatior*. Moreover, this area is approximately 70 km inland and has no typical Mediterranean plants whose pollen season coincides with *A. elatior* (23). However, about one third of our patients sensitized to *A. elatior* were inhabitants of Split and its surroundings, which supports the view that *A. elatior* pollen has spread as far as the sea coast. There is evidence that this plant can spread 6-20 km a year (1). *A. elatior* pollen grains have good aerodynamic properties and can migrate as far as 300 km. Every plant produces more than 60 000 seed grains, which can survive in the soil for more than 30 years, even in the freezing conditions (1). Climatic changes, global warming in particular, have influenced changes in the plant growth, distribution, and allergenic pollen emission (24). Increased CO_2 emission has particularly contributed to ragweed production and spreading (24). In Italy, the peak *A. elatior* pollen concentration in the air reached 700 grains/m^3 (7). In Poland, its concentrations increased from a few per cubic meter in a 24-hour air sample in the year 2000, to 98 grains/m^3 in 2002 (4). However, the proportion of *A. elatior* pollen in total pollen in Poland is still small, with the highest values in Krakow where this type of pollen accounts for 1.2% of total annual pollen production. During the pollen season, in some areas daily concen-

trations can exceed 50 pollen grains/m^3, and remain at this level for 4 to 6 weeks. This pollen concentration is considered sufficient to provoke symptoms of pollen allergy in 60%-80% of sensitized patients (3,10).

In the area around Lyon, France (25), ragweed pollen concentration increased from a few hundred grains per cubic meter in 1987 to 1200 grains/m^3 in 2002. Also, the number of days with pollen concentrations high enough to provoke pollen allergy symptoms in sensitized patients increased from 10 days in 1987 to 30 days in 2002.

In Croatia, clinically significant concentrations of *A. elatior* pollen were recorded throughout the northern inland areas, reaching their peak in the early September and declining from the east to the west (9-11,26). In Zagreb, the proportion of *A. elatior* pollen in total pollen counts during September and October usually reaches 80% (27).

These facts about pollen distribution and concentrations of *A. elatior* in Croatia raise concern, as the plant has a great allergenic potency and the number of sensitized persons is rising. In Hungary, 60% of patients with pollen allergy are sensitized to *A. elatior* (5), in Czech Republic 20% (6), in northern Italy and around river Rhone in France 12% (8,26). In North America, concern about ragweed allergy is even greater; ragweed pollen accounts for one third of the annual pollen production, and accounts for 50%-75% of all pollen allergies (28).

The results of this study are a continuation of our earlier studies, which evaluated pollen distribution and allergies in southern Croatia. It confirmed the presence of *A. elatior* pollen in the air around the town of Sinj and, to a lesser degree, Split, as well as its relation to pollen allergies in these areas. Although *A. elatior* pollen accounts for a maximum of 12% of total weekly pollen counts in these areas, our results showed that in the season almost half of the patients with symptoms of pollen allergy were sensitized to *A. elatior*, which confirms the great allergenic potency of this pollen. The pollen of *A. elatior* should be included in the routine diagnostic set of inhaled allergens in southern Croatia. This also calls for introducing measures for controlling the growth and spread of *A. elatior* in this area, particularly on uncultivated and neglected land.

Acknowledgments

The authors thank Ivana Maloča, MD, from the Institute for Medical Research and Occupational Health for technical assistance in the presentation of the results.

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