

Conference Paper

HYPERSENSITIVITY TO POLLEN ALLERGENS ON THE ADRIATIC COAST

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This paper describes a study of air concentrations of pollens and a calendar of pollination around the town of Split on the Croatian Adriatic in 1994. High pollen concentrations of *Parietaria officinalis* dominated during the year (up to 20 % from April to June) followed by the pollens of *Pistacia lentiscus*, *Olea europaea*, *Pinus halepensis*, *Juniperus oxycedrus*, *Acacia baileyana*, *Artemisia vulgaris*, *Ambrosia elatior* and *Cistus monspeliensis*. In 1994-95, skin prick tests using commercially available standard inhalation allergens and specially prepared pollen allergens were performed on 3,500 patients with allergic respiratory symptoms. About 30 % were allergic to standard pollen allergens (mixed grass pollen, mixed tree pollen, *Parietaria officinalis* and *Pittosporum tobira*). Hypersensitivity to more than one allergen was found in 45 % of patients, whereas 15 % did not react to any of the standard allergens. Additional testing with newly prepared individual allergens (*P. lentiscus*, *O. europaea*, *P. halepensis*, *A. baileyana*, *C. monspeliensis*, *A. vulgaris*, *A. elatior*) revealed hypersensitivity in a number of patients, but 36 % showed no reaction. This finding suggests that further studies of this kind are needed for additional identification, isolation, and characterisation of pollen allergens that are present in the Adriatic coast.

KEY WORDS: *airborne pollens, pollen calendar, pollen concentrations, reaction to allergens*

Most European countries have calendars of pollination providing information on the occurrence and duration of plant pollination in an area and the quantitative composition pollens (1-3). Measuring stations on the most important vegetation points in Croatia have been established since 1973, allowing a pollination calendar for continental and coastal regions to be made after years of follow-up (4).

The identification of plants, measurements of pollen air concentrations and plant pollination calendar for the town of Split were made during the 80s (5). To identify possible hypersensitivity and its relation to respiratory symptoms, our patients (patients with symptoms suspected to allergies) had the skin prick test with allergens prepared of pollens of the most typical plants in central Dalmatia (6). Recent years

are reported to have had longer pollination seasons, higher air pollen concentrations and the appearance of plant species that were new to a specific area worldwide (7). This is the reason why the number of hypersensitive persons and the number of new cases of pollinosis has increased. This prompted us to intensify the collaboration with local botanists in exploring the vegetation and identifying plants in the central Dalmatia, in determining the beginning and the duration of the pollination of every single plant species, and in measuring air pollen concentrations. This effort produced a new pollination calendar and made it possible to evaluate the importance of pollens found in causing hypersensitivity and respiratory symptoms in general population of the Central Dalmatia.

Table 1 General and clinical features of the study and control groups

Parameter	Study group N=3,500		Control group N=50	
	Men N=2,100	Women N=1,400	Men N=25	Women N=25
Age/years				
mean (range)	50(15-70)	42(16-72)	30(17-60)	31(18-65)
Symptoms duration/years				
mean (range)	25 (2-50)	20 (2-45)		
Familiar history/numbers of subjects				
positive	1,700	750		
negative	400	650		
Place of living/number of subjects				
town of Split	1,300	900	10	10
costal region	650	350	10	10
northern regions	150	150	5	5

SUBJECTS AND METHODS

Subjects

This study included 3,500 patients who came to the Department for Pulmonary Diseases in 1994-1995 (Table 1). Only those patients were included in the study who had experienced respiratory symptoms (rhinitis or asthma) for the minimum of two consecutive years. Conjunctivitis was found in about 40 % of the patients, and 10 % also had dermatitis. The study population consisted of 2,100 men aged between 15 and 70 years (mean age 50 years) and 1,400 women aged between

16 and 72 years (mean age 42 years). All patients lived in the town of Split or neighbouring areas which include the coastal region to the town of Ploče, the town of Trogir and adjacent areas, then the islands of Brač, Hvar, Šolta, Vis, and the inland area up to the towns of Sinj and Vrgorac. Most patients claimed that the symptoms intensified when they were staying out in the open, especially in the warmer seasons, and that the intensity of symptoms decreased when they would leave the area. Some patients, especially those whose history included hypersensitivity to the pollen of *Parietaria officinalis*, claimed that the vicinity of the plant, and especially the contact with it, caused respiratory symptoms. Most

Table 2 Hypersensitivity (skin prick test reaction) to various allergens in patients with respiratory symptoms (N=3,500) and in the control group (N=50) determined between 1994-1995

Allergen	Study group		Control group	
	n	%	n	%
Individual allergens				
House dust	930	26.6	6	12
<i>Dermatophagoides pteroniss.</i>	1,200	34.2	0	
Feathers	130	3.7	0	
Hemp	150	4.2	0	
Sheep wool	100	2.8	0	
Pollens (grass ¹ , trees ² , <i>P. officinalis</i> or <i>P. tobira</i>)	1,080	30.7	4	8
Molds	90	2.5	0	
Bacteria	90	2.5	0	
Animal hair	300	8.5	0	
Combined hypersensitivity	1,580	45.1	0	
Negative skin test	525	15.0	0	

¹Commercial product contains pollens of six grass species (see Materials and Methods)

²Commercial product contains pollens of nine tree species (see Materials and Methods)

n = number of patients with positive skin prick test

symptoms were observed over the warm season and showed a decreasing trend when the patient left the area. Patients with hypersensitivity to the pollen of *Ambrosia elatior* had similar reports. All patients had a skin prick test on the volar side of the arm using a set of standard inhalation allergens. In patients with negative reaction the test was repeated using pollens of various plants. The patients were not receiving corticosteroid or specific hyposensitivity treatment, nor had they been receiving antihistamines the week before the testing. The tested group included also 50 healthy patients with no personal and family history of atopies. They were tested for skin reactions in the same manner as the allergic patients (Table 2).

Allergens

All allergens were used as oily solutions. We used standard inhalation allergens (concentration 5000 PNU), including house dust, *Dermatophagoides pteronyssinus*, *P. officinalis*, *P. tobira*, hemp, sheep wool and allergen combinations (animal hair, feathers, moulds, bacteria, grass pollen, and tree pollen). Combined grass pollen allergen (used at the concentration of 5,000 PNU) contained pollens of six different grasses (*Poa pratensis*, *Phleum prtense*, *Secale cereale*, *Alopecurus pratensis*, *Agrostis alba* and *Dactylis glomerata*). Combined tree pollen allergen (used at a concentration of 5,000 PNU) contained pollens of nine trees (*Corylus avellana*, *Betula pendula*, *Sambucus nigra*, *Tilia cordifolia*, *Pinus nigra*, *Acacia*, *Salix alba*, *Populus alba* and *Platanus*). All patients were also tested for allergy to the pollens of *P. officinalis* and *Pitosporum tobira*.

Additional testing was performed using the pollen allergens of *Pistacia lentiscus*, *Olea europaea*, *Cistus monspeliensis*, *Pinus halepensis*, *Juniperus oxycedrus*, *Acacia baileyana*, *Ambrosia elatior*, and *Artemisia vulgaris*, all at a concentration of 5,000 PNU. In every testing we also applied histamine chloride solution (1 mg/mL) as the positive control and glycerin solvent as the negative control.

Skin prick tests

Skin testing with allergens was performed on the volar side of the arm using a 1 mm lancet under an angle of 60-70 degrees. The patients were not informed about the type of allergens applied. The reaction was measured 15-20 min after testing, and was considered positive if the urticarial wheal

was 3 mm or more in diameter, taking also in consideration the negative control reaction. The urticarial 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".

Specific IgE antibodies

Specific IgE antibodies for the pollens of *O. europaea*, *P. officinalis*, *P. halepensis*, *A. elatior*, *A. vulgaris* were determined using immunological CAP technology, UNI CAP 100 (Pharmacia AB Diagnostic, Uppsala, Sweden). Values under 0.35 kU/L were considered unspecific and those greater than 0.35 kU/L increased (8).

Determination of pollen concentrations in the air

Pollen concentrations in the air were determined using the gravimetric method. Glass slides were distributed to several locations in the town of Split and its suburbs. The slides were read on a weekly basis throughout the year. The results were presented as the percentages of individual pollens in the total pollen found on the glass slide (9).

RESULTS

The flowering pattern of a number of plants common in the central part of southern Croatia is shown in Figure 1. It can be noticed that the period of *Parietaria officinalis* pollination is very long with its peak in spring; it stops during July and August and starts again in mid-August and lasts through September and October. The air concentration of



Figure 1 Pollination calendar for the town of Split and its suburbs (White areas - gradual pollen appearance and disappearance; black areas - periods with the highest pollen concentrations).

Table 3 Hypersensitivity to individual pollens in patients who were positive (N=100) and negative (N=150) to combined pollen allergens
The results are presented in percentages of tested patients. The percentages were obtained by testing a sample of 250 patients of whom 100 were randomly selected from a group of 1,100 patients with positive skin prick test to standard pollen allergens and 150 from a group of 525 patients with negative skin prick test to pollen allergens (see Table 1)

Allergen	Patients (%) with positive skin test to pollen allergens				Total
	Grass	Tree	Parietaria off.	None	
Grass	6	-	1.2	-	7.2
Tree	-	4	-	-	4
<i>Parietaria off.</i>	4.8	-	20	-	24.8
<i>Pitospora t.</i>	-	-	-	-	4
<i>Olea e.</i>	2.6	-	-	7	9.6
<i>Pinus h.</i>	-	-	-	8.2	11.2
<i>Juniperus o.</i>	-	-	-	7.6	9.6
<i>Acacia b.</i>	-	1.2	-	6.0	7.2
<i>Cistus m.</i>	2	-	-	10	18
<i>Pistacia l.</i>	-	-	-	12	18
<i>Artemisia v.</i>	-	-	-	12	12
<i>Ambrosia e.</i>	-	-	-	14.4	14.4
None	-	-	-	36	36

Parietaria pollens dominated during the year, with its peak during April and May when it amounts up to 20 % of all pollens. Pollens of *Pistacia lentiscus*, *Olea europaea*, *Pinus halepensis*, *Juniperus oxycedrus*, *Acacia baileyana*, *Cistus monspeliensis*, *Ambrosia elatior*, *Artemisia vulgaris*, *Amaranthus maritimus*, *Laurus nobilis*, *Por. Ericaceae*, were found in lower concentrations and for shorter time periods.

The results of skin prick tests indicate that 1,200 of 3,500 patients (34.2 %) were positive to *Dermatophagoides pteronissinus*, 930 (26.6 %) to house dust, and 1,080 (30.7 %) to standard pollen (combined tree and grass pollen allergen; *Parietaria off.* and *P. Tobira*). Furthermore, 1,580 patients (45.1 %) had hypersensitivity to combined allergens, and 525 patients (15 %) with respiratory symptoms were negative to all tested allergens.

Further analysis focused on subjects with a history of seasonal pollen rhinitis (SPR) and asthma (SPA). One hundred patients were randomly selected from the group of patients who were hypersensitive to standard inhalation allergens and 150 from the group whose hypersensitivity tests to the same allergens were negative. They were tested with newly isolated pollen allergens that are characteristic for our region, and whose air concentration was measured. The data obtained reveal the total proportion of hypersensitivity to newly isolated allergens of this area (Table 3, far right column). The first three columns of the

same table show cross-reactivity between tested allergens. Skin prick test with new allergens led to accurate diagnosis in about 64 % of patients who were negative to standard allergens and had SPR and SPA. However, in 36 % of patients we did not find the allergen that was causing hypersensitivity (Table 3, fourth column).

In addition, our study revealed several cross-reactivities between individual allergens. Some 75 % of patients allergic to *J. oxycedrus* were also

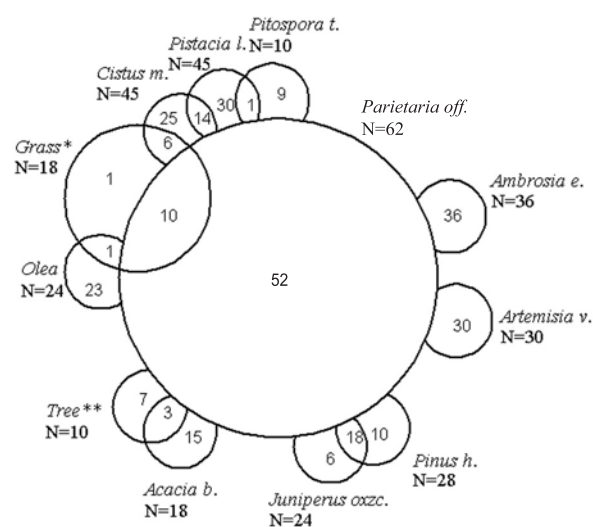


Figure 2 Skin prick test distribution among 250 patients with SPR (seasonal pollen rhinitis) and SPA (seasonal pollen asthma) (*commercial products which contain six grass allergens, ** commercial products which contain nine tree allergens, see Materials and Methods).

reactive to the pollen of *P. halepensis*. At the same time, 64 % of patients allergic to *P. halepensis* were also allergic to the pollen of *J. oxycedrus*. Patients hypersensitive to *P. lentiscus* showed cross-reactivity to the pollen of *P. tobira* (2 %) and *C. monspeliensis* (31 %). Thirty one percent of patients hypersensitive to *C. monspeliensis* were also positive to the pollen of *P. lentiscus*. No cross-reactivity was found between *P. tobira* and *C. monspeliensis*. Patients hypersensitive to *A. elatior*, *A. vulgaris* and *P. officinalis* in most cases did not show any cross-reactivity (Figure 2).

The concentrations of IgE antibodies specific for *Parietaria off.*, *Ambrosia e.*, *Artemisia v.*, *Pinus h.*, *Olea e.* and *Juniperus o.* were measured in patients who were hypersensitive to those allergens. We found a significant correlation between the mean diameter of the wheal and the serum concentration of IgE antibodies for the same allergen (Table 4).

Table 4 Comparison of the skin prick test results with the concentrations of specific IgE

Allergen	Skin prick test $\bar{x} \pm SD$ (mm)	Specific IgE $\bar{x} \pm SD$ (kU/L)	Correlation coefficient R	P-value
<i>Parietaria off.</i>	12±2	60.3±7.2	0.84	<0.001
<i>Ambrosia e.</i>	7±2	7.3±2.4	0.66	<0.01
<i>Artemisia v.</i>	8±3	6.2±1.4	0.65	<0.01
<i>Pinus h.</i>	8±2	6.3±2.2	0.65	<0.01
<i>Olea e.</i>	8±2	8.4±3.2	0.73	<0.001
<i>Juniperus o.</i>	7±2	5.3±2.1	0.64	<0.01

DISCUSSION

Wind-pollinated plants are potent allergens due to the great dispersal of their pollen grains (10). The most extensive scientific works in that area, document that plants which grow on uncultivated soil are the greatest sources of pollens (11). Air concentrations of pollen for some Mediterranean plants were determined, the existence of hypersensitivity was investigated and its correlation with the presence of symptoms was also found. The *Urticaceae* species are spread as weed in the Mediterranean countries (12, 13), including Croatia (14), and along the Atlantic Ocean coast (15). But one of most represented species is *Parietaria officinalis* which is an annual wind-pollinated plant,

that grows on calcareous soil, and which is of great significance because it flowers during almost entire year and produces enormous quantities of pollens (Figure 1). At the time of peak pollination from April to June, the concentration of pollen in the air was 20 % and 62 patients with hypersensitivity to *Parietaria off.* (24.8 %) related their symptoms to its flowering (they claimed that the vicinity of the plant would cause strong nasal reaction, sneezing, and tears). During mild winters it starts producing pollens as early as in February (up to 8 %) and it flowers regularly again in September and October (up to 6 %). No significant cross-reactivity with other plant species was found, which indicates its allergenic specificity (16). Only 10 hypersensitive patients (4.8 %) showed hypersensitivity to grass pollens too (Table 3). The majority lived in the suburbs where *Parietaria* grows on uncultivated soils. The correlation between air concentrations of *Parietaria off.* and hypersensitivity symptoms development were similar to our results from ten years ago (5).

Species *Cistaceae*, especially *Cistus l.*, is very common in uncultivated grounds in Dalmatia. *Cistus monspeliensis*, *Cistus salviaefolius*, and *Cistus creticus* dominate that area. They grow mostly at the seashore, among olives, and produce a lot of pollen. But today they can also be found inland. This species flowers from April to June (Figure 1) and uses wind and insects for pollination. The medium concentration of *C. monspeliensis* pollen in suburbs amounted to 14 %. We found hypersensitivity to *C. monspeliensis* in 18 % of the patients tested (Table 3). All hypersensitive patients who lived in the suburbs and on islands reported symptoms during that time of year. Only a small part of them (31 %) also showed hypersensitivity to *Pistacia l.* at the same time. These results do not differ significantly from the ones reported earlier (5).

Another bush that is very common in Dalmatia region is *Pitosporum tobira*. It can be found in towns because it is usually grown for hedges or decoration. It has a long pollination period, from end March to June, but produces little pollen (up to 5 %). Therefore only 4 % of patients resulted hypersensitive to *Pitosporum t.*. This allergen has not yet been described in relevant literature.

Israeli authors have described the *Pistacia* tree genus as a very important source of respiratory hypersensitivity (17). In Croatia there are no data about hypersensitivity to that allergen. It grows throughout the Mediterranean. Southern Croatia is dominated by *P. lentiscus*, which in March (sometimes as early as February) starts producing enormous amounts of pollen and continues until the

end of April. Maximum recorded air concentration amounted to 13 %. Of 250 patients with SPR and SPA, 18 % were hypersensitive to *P. lentiscus* (Table 3), and their symptoms corresponded with its flowering. Most hypersensitive patients (70 %) lived in the suburbs of Split, and less (25 %) in the town itself. A few patients had concomitant hypersensitivity to *C. monspeliensis* and *P. tobira*.

Oleaceae genus, especially *Olea l.* is very important allergen (18). In our area there are two species: *Olea europaea* and *Olea oleaster* (domesticated and wild type, respectively). They are widespread in dry and rocky Dalmatian terrains and in the whole Mediterranean. *Fraxinus*, *Ligustrum* and *Syringa* species belonging to the same family are also widespread in our country. The most important allergen from the *Oleaceae* family is pollen of *Olea Europaea* (18). It flowers from April to June (Figure 1) and produces great amounts of very allergenic pollen, so the percentage of hypersensitive people is reasonably high. Reports about its allergenic potentials come from many Mediterranean countries (19). Medium pollen concentrations in our region were 18% in June, when hypersensitive persons mostly complained of respiratory symptoms. All patients reported that they had olive trees in their gardens and noticed that the presence of respiratory symptoms mostly coincided with olive tree flowering. Of 250 patients with SPR and SPA, 9.6 % were hypersensitive to the olive tree pollen (Table 3), which documents its high allergenic specificity. All patients lived in the suburbs of Split.

In the studied area *Juniperus oxycedrus*, from the Cupressaceae family flowers from February to June. It is an evergreen tree or bush that produces large amounts of pollen which is carried by insects or wind. It grows in the Mediterranean and sub-Mediterranean regions. *Juniperus communis* and *Cupressus sempervirens* are two species from the same Cupressaceae family that can be found in Dalmatia and eastern Mediterranean regions. The peak concentration was measured in March and the average concentration was 14 %. Of 250 patients with SPR and SPA, 9.6 % were hypersensitive. The majority (75 %) of these also reacted to the pollen of *Pinus h.* and they all lived in the suburbs of Split.

Pinaceae family is widespread in Dalmatia, and the species *Pinus halepensis* is the most common among pine tree species. It flowers from February to April and produces considerable amounts of pollen. The average air concentration is 13 % (Figure 1). *Pinus nigra* and *Pinus pinea*, also found in Dalmatia, are important

because they belong to the same family which can lead to cross-reactivity. Of 250 patients with SPR and SPA, 10 % were hypersensitive. Most of them (64 %) were also hypersensitive to the pollen of *Juniperus oxycedrus*. Hypersensitive patients manifested severe symptoms of pollinosis. The air concentration and the percentage of hypersensitive patients correspond to earlier findings (5).

The *Fabaceae* family is present only in the southern Croatian through *Acacia baileyana*. The results showed that its air pollen concentration could reach 10 % as early as January, and it remained at that level until the end of April. *Robinia pseudoacacia* is a plant that belongs to the same family and it grows further inland. It is possible for people who live in the coastal area to develop allergic reactions when travelling there. In the group of 250 hypersensitive patients, 7.2 % were positive to *Robinia p.*

Great air concentrations of the pollens of laurel (*Laurus nobilis*) (average 15 %) and *Ericaceae* were measured from February to the end of June. *E. arborea* and *multiflora* dominate in our regions, and they flower from January to May and again from October to the end of November. These species produce great amounts of pollens, with the average concentration reaching 10 %. *Chenopodium spp.* was also found in notable air concentrations. Testing for these allergens is now under way.

Over the last few years, studies from many countries have shown that the number of patients hypersensitive to weed pollens is on a continuous rise because these allergens are produced in great quantities, mostly in late summer and early autumn. The same is true for the number of patients hypersensitive to grass and tree pollens, who have symptoms throughout the year.

Artemisia l., from *Asteraceae* family, dominates in our coastal region. *Artemisia vulgaris*, also very common in this area, starts flowering in April, dominates through May and June, and again in the second half of August to October. The average air concentration amounted to 10 %. Our earlier studies did not find hypersensitivities to this allergen, but this study (1994-1995) identified 12 % positive reactions to *Artemisia v.* There are several reports in literature about hypersensitivity to *Artemisia* pollens and its treatment (20).

Ambrosia (ragweed) is the number one allergen in North America (21) and has been spreading very fast to northern and western Europe (22, 23). The

latest reports show that it has reached Sardinia (7). A great number of patients who are hypersensitive to ragweed live in northern Croatia. Earlier studies of the Split area did not identify ragweed pollens (5). This study identified minor air concentrations of *Ambrosia l.*, and especially *Ambrosia elatior* as early as April, whereas great concentrations were found in May and June and again in August and September. The average pollen air concentration was 10 %. Of the examined patients with SPR and SPA, 14.4 % were hypersensitive and had severe symptoms during ragweed flowering. This study documented the presence of ragweed on uncultivated land near roads and railways where it got during the transportation of animals from the north. Ragweed causes respiratory problems which correspond to exposure. Patients can identify ragweed because it has a specific look and grows in particular places. Lately it has been growing in new, unfinished urban quarters, so among many hypersensitive patients that mostly live in suburbs there are some that live in newly urbanized regions.

Our study has shown that grass, tree and bush pollination have not changed in duration and pollen air concentrations over the last ten years. Consequently, the incidence of relevant allergies has not changed either. On the contrary ragweed (*Ambrosia*) and *Artemisia* pollen air concentrations are increasing and so are the respective incidences of allergic patients. Further identification of new allergens in Dalmatia is needed, because the causes of SPR in many persons are still unknown.

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Sažetak

PREOSJETLJIVOST NA ALERGENE PELUDA BILJAKA U PODRUČJU OBALE JADRANSKOGA MORA

U središnjem dijelu Južne Hrvatske, u priobalnom području Jadranskog mora, sa središtem u gradu Splitu, određivali smo koncentraciju peluda u zraku tijekom 1994. godine i izradili peludni kalendar.

Tijekom cijele godine u zraku je dominirala visoka koncentracija peluda crkvine (*Parietaria officinalis*), do 20 % od travnja do lipnja. Peludi *Pistacia lentiscus*, *Olea europea*, *Pinus halepensis*, *Juniperus oxycedrus*, *Acacia baileyana*, *Artemisia vulgaris*, *Ambrosia elatior* i *Cistus monspeliensis* nađeni su u nižim koncentracijama i u kraćem razdoblju. Kožni test urađen je u 3500 osoba tijekom 1994./95. godine, koje su imale alergijske smetnje dišnih organa, a upotrijebljeni su standardni komercijalno pripremljeni inhalacijski alergeni i posebno za tu svrhu izrađeni alergeni peludi biljaka mediteranskog podneblja. Oko 30 % pacijenata bilo je preosjetljivo na standardne inhalacijske alergene peluda (mješavina alergena peluda trava, mješavina alergena peluda stabala *Parietaria officinalis*, *Pitospora tobira*). Kod 45 % pacijenata nađena je preosjetljivost na više od jednog alergena, 15 % pacijenata nije reagiralo ni na jedan od standardnih alergena. Dodatna testiranja novoproduzvenim alergenima biljaka (*Pistacia lentiscus*, *Olea europea*, *Pinus halepensis*, *Acacia baileyana*, *Cistus monspeliensis*, *Artemisia vulgaris* i *Ambrosia elatior*) otkrila su nam preosjetljivost kod velikog broja pacijenata, ali i nakon toga 36 % pacijenata nije reagiralo ni na jedan od testiranih alergena. Ova informacija potvrđuje potrebu za daljnjim istraživanjima ove vrste, za dodatnom identifikacijom, izolacijom i karakterizacijom alergena peluda u području obale Jadranskog mora.

KLJUČNE RIJEČI: kalendar polena, koncentracije polena, poleni u zraku, reakcije na alergene

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