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# Aerobiology of Sweet Chestnut (*Castanea sativa* Mill.) in North-West Croatia

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**A B S T R A C T**

*The aims of the study were to analyse characteristics of the Castanea airborne pollen and to compare aeropalynological data obtained from two sampling stations in north-west Croatia. The study was conducted in Zagreb and Samobor during the 2003–2006 periods, using the seven-day volumetric samplers of the Hirst design. In both study areas, the seasons of chestnut pollination were similar and lasted from June to the end of July, which is comparable to other European cities. A general rule was noticed – the shorter the main pollen season, the higher the pollen peak concentration. Although the pollen season of Fagales pollen is prolonged to summer in the area of inland west-north Croatia due to the genus Castanea summer pollination, the number of days with pollen air concentration higher than 50* per *$m^3$ was low and was not likely to have any major effects in allergic individuals. Airborne pollen concentration of Castanea showed positive statistically significant correlation with air temperature and negative non-significant correlation with precipitation. Because of the non-significant differences between the two stations, for a possible long-term forecast model for Fagales airborne pollen for this part of north-west Croatia, aerobiological data obtained from only one station are sufficient.*

***Key words:*** *aerobiology, Castanea sativa airborne pollen, north-west Croatia, Zagreb, Samobor*

## Introduction

Sweet chestnut, *Castanea sativa* Mill., belongs to the family Fagaceae Dumort of the order Fagales Engl. that is widespread in the temperate zone of the northern hemisphere. Deciduous sweet chestnut tree can attain a height of more than 30 m, has a large and exuberant crown, and can be up to 500 years old. Male flowers form clusters (of three or more) in upright catkins 10–30 cm long. Female flowers are located at the base of male catkins, forming clusters of 3–7 flowers within common sheath. While other allergenic genera of the family Fagaceae produce pollen grains during the spring and early summer months[1], e.g. *Fagus sylvatica* L. (beech) and various taxa of the genus *Quercus* (oak), sweet chestnut blossoms in June when it's leaves are fully developed[2,3]. The pollination is entomophilous and anemophilous. Because of the abundant pollen production (several billion grains *per* tree) and small size of each grain, pollen of the genus *Castanea* has been used as a marker in studies of medium-range and long-range pollen transport[4]. Besides pollen, which is held in very high esteem because of it's medicinal properties, sweet chestnut produces large amounts of nectar. The sweet chestnut fruits are edible nuts used in human diet since ancient times because of their high nutritive value[5].

It is believed that sweet chestnut was naturally spread across the six large refugial regions worldwide (including Balkan Peninsula) after glaciations, subsequently spreading beyond its natural areal thanks to cultivation towards the end of the Roman Empire[5]. Nowadays, it is widely spread over large parts of Europe and in north Africa[2]. In Croatia, sweet chestnut grows as a native species on Medvednica Mountain, Žumberak-Samobor Mountains, in Banovina and Istria[6]. Minor chestnut woods are found on the island of Cres[7]. Chestnut woods (Ass. *Querco-Castanetum sativa*/Ht. 1983) develop on acid soil (pH

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4–5), at warm habitats, on plateaus and mild slopes, at 250–550 m above the sea level, and do not tolerate contaminated atmosphere. Sessile oak (*Quercus petraea* Liebl.), hornbeam (*Carpinus betulus* L.) and beech (*Fagus sylvatica*) can be found in the tree layers, along with sweet chestnut. In the last decade, chestnut habitats have been considerably reduced, leading to its disappearance from Croatian forests, most probably due to the parasitic fungus *Cryphonectria parasitica* (Murr.) Barr., which causes chestnut bark cancer. This parasitic fungus is very aggressive and can cause tree infection irrespective of their age and vitality. In Croatia, the disease has been first recorded in 1950[8].

According to the Köppen's classification of climate types, Zagreb and Samobor regions belong to a type of continental moderately warm rainy climate[9].

It has been noticed that different processes of cross-sensitivity are frequent among the three most common families of the order Fagales, i.e. Betulaceae, Corylaceae and Fagaceae, because subjects sensitive to Betula pollen antigens show positive reactions to antigens of other genera belonging to the related families[10]. Therefore, the genera of the Fagaceae family play a supporting role in inducing pollen allergies in Europe. Thus, *Castanea*, *Fagus* and *Quercus* can only be responsible for allergic manifestations when airborne pollen grains are especially abundant[11]. However, *Castanea* pollen has been classified as an allergen by some authors[12–14], particularly in some areas or countries, e.g., Switzerland and France, where studies have indicated that a high percentage of the population is sensitive to this pollen (37% in Locarno, Switzerland, and 35% in Paris, France)[4].

North-west Croatia is known as a region rich in all Fagales taxa[2,15], but only a little data on the allergenic effects of the *Betula*, *Corylus* and *Alnus* pollen in Zagreb have been reported[16] so far. Since the time of allergic symptoms in individuals sensitive to pollen of the order Fagales can be prolonged due to the summer pollination of the genus *Castanea*, there is a need for information on the allergenic influence of the Fagales taxa in inland Croatia. Unfortunately, there hasn't been any published data about any particular pollen allergy of the Croatian population so far.

Furthermore, it is known that different aerobiological stations, situated in close proximity, could have significant correlation between daily pollen concentrations which indicates that pollen data from only one station could be sufficient for long-term forecast for similar areas[17–19].

Thus, one of the aims of the present study was to analyze pollen season start and characteristics of the *Castanea* airborne pollen in inland Croatia as a region relatively rich in chestnut woods, with the purpose to contribute to the clarification of the occurrence of symptoms in individuals allergic to pollen of the Fagales taxa in the inland north-west Croatia. The other was to compare aeropalynological data obtained from two sampling stations, in order to give basis for future forecast for the same region.

## Materials and Methods

This aeropalynologic study was carried out in Zagreb and Samobor (Figure 1) in vegetation seasons during the 2003–2006 periods. Both cities are located in inland

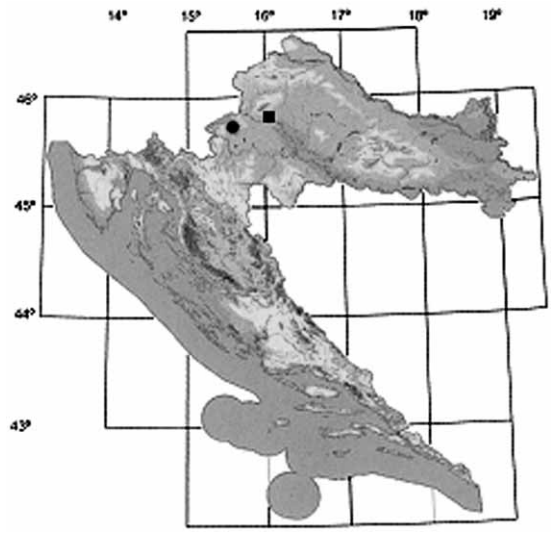


*Fig. 1. Geographical positions of the sampling sites Zagreb (■) and Samobor (●) in north-west Croatia.*

**TABLE 1**
AVERAGE MONTHLY AIR TEMPERATURES AND PRECIPITATIONS IN ZAGREB AND SAMOBOR, 2003–2006

| | | Temperature (°C) | | Precipitation (mm) | |
|---|---|---|---|---|---|
| | YEAR | JUNE | JULY | JUNE | JULY |
| ZAGREB | 2003 | 23.9 | 23.0 | 65.6 | 62.3 |
| | 2004 | 19.1 | 21.1 | 102.2 | 69.7 |
| | 2005 | 19.9 | 21.5 | 68.6 | 137.1 |
| | 2006 | 20.5 | 23.8 | 40.3 | 31.7 |
| SAMOBOR | 2003 | 24.3 | 22.9 | 36.8 | 99.8 |
| | 2004 | 19.2 | 21.4 | 83.6 | 71.3 |
| | 2005 | 20.3 | 21.5 | 93.7 | 213 |
| | 2006 | 20.7 | 23.6 | 48.8 | 93.6 |

Croatia (Figure 1), provide data on chestnut pollen from the surrounding Medvednica Mountain and Žumberak-Samobor Mountain, respectively, which are the most representative localities of sweet chestnut in Croatia.

The City of Zagreb, the capital of Croatia, is situated on the Medvednica hillsides extending to up to 1035 m above the sea level and along the Sava River. A major part of the city is situated at 112 m above the sea level, and according to geographical coordinates at $45^0$15'N and $15^0$30'E. The northern part of the city is greatly influenced by the Medvednica Nature Park with its plant life predominated by forests (63.6%). Forest vegetation mostly includes the following species: *Acer campestre* L., *A. pseudoplatanus* L., *A. platanoides* L., *Ulmus glabra* Hudson, *Fraxinus excelsior* L., *Carpinus betulus*, *Ostrya carpinifolia* L., *Corylus avellana* L., *Fagus sylvatica*, *Quercus petraea* (Mattuschka) Liebl., *Taxus baccata* L., *Abies alba* L., *Ilex aquifolium* L., *Castanea sativa*, *Tilia cordata*, different taxa of the genus *Rubus*, *Sambucus nigra* L., R*obinia pseudoaccacia* L., etc. Ruderal plants and weeds with a considerable proportion of alien plants prevail in the east, west and south areas along the city outskirts. Many species growing in numerous city parks also release pollen grains into the atmosphere[20].

The town of Samobor is situated at $45^0$48'N and $15^0$43'E, and 168 m above the sea level, about 30 km south-west from Zagreb, surrounded by the Žumberak and Samobor Mountains as a Nature Park of 350 $km^2$, extending from 180 to 1178 m above the sea level. The town of Samobor is rich in woods, which occupy 44% of its territory[15]. These woods are predominated by similar species as those in Zagreb: *Acer campestre*, *A. pseudoplatanus*, *A. platanoides*, *Carpinus betulus*, *Corylus avellana*, *Fagus sylvatica*, *Quercus petraea*, *Ulmus glabra*, *Castanea sativa* and *Taxus baccata*. However, ruderal plants and weeds are less common[16]. Sessile oak (*Quercus petraea*) along with sweet chestnut (*Castanea sativa*) grows on southern and south-west exposures of the Samobor Mountain, mostly on silicate ground, argillaceous and sandy soil poor in lime, forming the *Querco-Castanetum sativa* Ht. 1938 association[15].

Meteorological data on the two locations (Table 1) were supplied by the Croatian Meteorological and Hydrological Service.

The seven-day volumetric samplers of the Hirst design[21] were placed in southern part of the City of Zagreb (45°46' N and 15°58' E, 157 m above the sea level, 14 m above the ground) and in the centre of Samobor (45°48' N and 15°43' E, 168 m above the sea level,18.5 m above the ground). The sampler absorbs 10 L of air *per* minute through the 2x14 mm opening protected from precipitation, which corresponds to the average human inspiration. The device can rotate freely by using an inbuilt wing, depending on the wind flow, so that the air absorbing opening is always turned to the wind. The cylinder is connected to a timer and rotates at a rate of 2 mm/h or 48 mm/day, allowing for determination of pollen concentration at 2-hour intervals. The cylinder carries a transparent polyester tape coated with a sticky substance (silicon oil). Airborne particles bump against the tape surface and stick to it. The tape was removed once a week, cut to a length corresponding to 24-hour pollen sampling, applied onto a glass slide and embedded in Gelvatol medium. The slides were examined under a light microscope at magnification x400, according to the Europaean Aeroallergen Network (EAN) recommendations. Microscopic slide analysis included identification and counting of pollen grains by the method of longitudinal lines[22]. Results were expressed as the mean daily pollen concentration (pollen count *per* $m^3$ air *per* day), and classified as nil, low, moderate, and high[23]. These terms refer to pollen count thresholds required for a small, medium or large percentage of the susceptible population to develop symptoms associated with the presence of this type of pollen[23]. The onset of the main pollen season was defined as the first day with at least 1 pollen grain/$m^3$ air recorded, followed by consecutive days with ≥1 pollen grain/$m^3$ air[24,25]. The first of five consecutive days free from pollen grains was considered as the end of the main pollen season[26].

Relationships between *Castanea* pollen concentration and meteorological features were calculated by the Pearson's rank correlation. To study the association between the daily pollen concentrations measured in Zagreb and Samobor stations, the Spearman's correlation test was carried out. For statistic analysis the SPSS 13.0 (2004) software was used.

## Results

The aerobiological study of sweet chestnut, *Castanea sativa*, conducted at two inland localities in northwest Croatia, revealed that the sweet chestnut pollen seasons in the 2003–2006 periods occur in June and July (Table 2). In the study period, the average chestnut main pollen season in Zagreb was 44 days long. The shortest pollination season of 35 days was recorded in 2003 while the longest pollination season of 54 days in 2004. For all study years, the main pollen seasons were in June. In Samobor, the average chestnut main pollen season was 41 days long. The shortest pollination season of 34 days was recorded in 2003 and the longest pollen season of 59

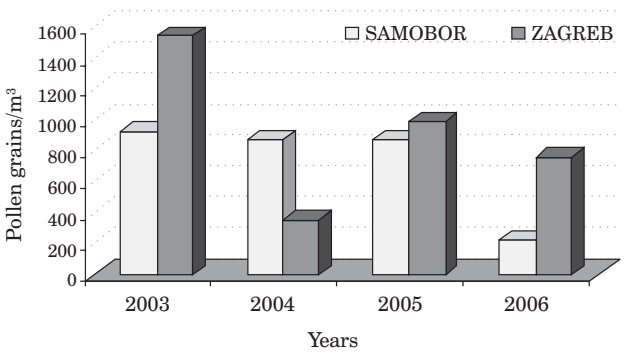


*Fig. 2. Annual pollen concentrations of* Castanea sativa *in Zagreb and Samobor, 2003–2006.*

**TABLE 2**
CHARACTERISTICS OF THE *CASTANEA SATIVA* POLLEN SEASONS IN ZAGREB AND SAMOBOR, 2003–2006

| | YEAR | Period of occurrence | Duration (days) | Annual total | % of total pollen count | Peak | Peak day | % June | % July | Days >50 |
|---|---|---|---|---|---|---|---|---|---|---|
| ZAGREB | 2003 | 05.06.–10.07. | 35 | 1546 | 7 | 321 | 14.06. | 51 | 2 | 9 |
| | 2004 | 08.06.–31.07. | 54 | 354 | 1 | 32 | 30.06. | 14 | 12 | 0 |
| | 2005 | 11.06.–22.07. | 42 | 986 | 3 | 172 | 29.06. | 29 | 6 | 5 |
| | 2006 | 16.06.–29.07. | 44 | 760 | 2 | 170 | 28.06. | 25 | 7 | 5 |
| AVERAGE | | | 44 | 912 | 3 | | | | | |
| SAMOBOR | 2003 | 05.06.–08.07. | 34 | 924 | 3 | 118 | 15.06. | 60 | 6 | 5 |
| | 2004 | 02.06.–30.07. | 59 | 877 | 4 | 105 | 28.06. | 20 | 31 | 4 |
| | 2005 | 16.06.–21.07. | 37 | 871 | 13 | 182 | 10.07. | 45 | 45 | 5 |
| | 2006 | 16.06.–20.07. | 35 | 222 | 2 | 73 | 20.06. | 29 | 11 | 1 |
| AVERAGE | | | 41 | 724 | 6 | | | | | |

days in 2004. The main pollen seasons in 2003 and 2006 were in June, and in 2004 and 2005 in July (Table 2).

The total pollen index of *Castanea* during the study period for the region of Zagreb (Table 2, Figure 2) was 3646 grains/$m^3$, with an average of 912 grains/$m^3$, accounting for 3% of total pollen spectrum. The highest annual pollen count of 1546 grains/$m^3$ (7% of total pollen spectrum) was recorded in 2003, and the lowest annual pollen count of 354 grains/$m^3$ (1% of total pollen spectrum) in 2004 (Table 2). The maximal daily pollen concentration of 321 grains /$m^3$ was recorded on June 14$^{th}$, 2003, while in 2004 the maximal daily pollen concentration was only 32 grains/$m^3$, registered on June 30, 2004. Daily pollen concentration higher than 50 pollen grains/$m^3$ was noticed in 2003 (9 days), 2005 (5 days) and 2006 (5 days).

The total pollen count of *Castanea* for the area of Samobor (Table 2, Figure 2) was 2894 grains/$m^3$, with an average of 724 grains/$m^3$ (6% of total pollen spectrum). The highest annual pollen count of 924 grains/$m^3$ was recorded in 2003, which accounted for 3% of total pollen spectrum. The lowest annual pollen count of 222 grains/$m^3$ was recorded in 2006, which accounted for 2% of total pollen spectrum (Table 2). The maximal daily pollen concentration of 182 grains/$m^3$ was recorded on July 10$^{th}$, 2005, while in 2006; the maximal daily pollen concentration was only 73 grains/$m^3$, registered on June 20$^{th}$. Daily pollen concentration higher than 50 pollen grains/$m^3$ was recorded in 2003 and 2005 (5 days both), and in 2006 (one day).

Correlations with meteorological parameters, calculated by the non-parametric Pearson's correlations index, were expressed for each locality and year, both for the whole chestnut pollination seasons (Table 3) and for the pre-peak seasons (Table 4). The correlations between daily pollen concentration and temperature (mean, minimum and maximum) in both calculations (Tables 3 and 4) were positive and significant for both locations and all

**TABLE 3**
PEARSON'S CORRELATIONS INDEX FOR THE WHOLE *CASTANEA SATIVA* POLLEN SEASONS ACCORDING TO THE METEOROLOGICAL PARAMETERS IN ZAGREB AND SAMOBOR, 2003–2006

| | | 2003 | 2004 | 2005 | 2006 |
|---|---|---|---|---|---|
| Zagreb | T mean | 0.254* | 0.501** | 0.272* | 0.328** |
| | Tmax | 0.201 | 0.491** | 0.324* | 0.263* |
| | Tmin | 0.324* | 0.392** | 0.207 | 0.391** |
| | Precipitation | –0.055 | –0.078 | –0.159 | –0.173 |
| Samobor | T mean | 0.281* | 0.112 | –0.009 | 0.439* |
| | Tmax | 0.237* | 0.135 | –0.026 | 0.171 |
| | Tmin | 0.313** | 0.093 | –0.093 | 0.218 |
| | Precipitation | 0.015 | –0.001 | 0.034 | –0.069 |

*Significance p=0.05
** Significance p=0.01
Tmax – maximal temperature
T mean – mean temperature
Tmin – minimal temperature

**TABLE 4**
PEARSON'S CORRELATION INDEX FOR THE PRE-PEAK *CASTANEA SATIVA* POLLEN SEASONS ACCORDING TO THE METEOROLOGICAL PARAMETERS IN ZAGREB AND SAMOBOR, 2003–2006

| | | 2003 | 2004 | 2005 | 2006 |
|---|---|---|---|---|---|
| Zagreb | T mean | 0.254* | 0.501** | 0.272* | 0.328** |
| | Tmax | 0.201 | 0.491** | 0.324* | 0.263* |
| | Tmin | 0.324* | 0.392** | 0.207 | 0.391** |
| | Precipitation | –0.055 | –0.078 | –0.159 | –0.173 |
| Samobor | T mean | 0.281* | 0.112 | –0.009 | 0.439* |
| | Tmax | 0.237* | 0.135 | –0.026 | 0.171 |
| | Tmin | 0.313** | 0.093 | –0.093 | 0.218 |
| | Precipitation | 0.015 | –0.001 | 0.034 | –0.069 |

* Significance p=0.05
** Significance p=0.01
Tmax – maximal temperature
Tmin – minimal temperature
T mean – mean temperature

**TABLE 5**
SPEARMAN'S CORRELATION COEFFICIENTS (r) BETWEEN DAILY *CASTANEA SATIVA* POLLEN CONCENTRATIONS IN ZAGREB AND SAMOBOR, 2003–2006

| | 2003 | 2004 | 2005 | 2006 | Average |
|---|---|---|---|---|---|
| r | 0.696** | 0.537** | 0.657** | 0.603** | 0.510** |

** Significance p≤0.01

years. However, in Samobor, this correlation was not significant in 2004 and 2005, when it was negative. Non-significant correlations between pollen concentrations and rainfall were obtained in both tests and localities for all study years (Tables 3 and 4). Otherwise, these correlations were negative. Exceptions were slightly positive but also non-significant correlations observed in Samobor in 2003 and 2005. The Spearman's correlation test between the daily pollen concentrations on both aerobiological stations was positive and highly significant (at p≤0.01) for all years and consequently for average of all four years studied (Table 5), which indicates a high association between pollen concentrations in Zagreb and Samobor.

## Discussion and Conclusion

During the 4-year (2003–2006) pollen monitoring in inland northwest Croatia, airborne pollen of sweet chestnut (*Castanea sativa*) was present in the atmosphere of the City of Zagreb and Samobor throughout the summer months June and July (Table 2). Such main pollen seasons are in accordance with data of *Castanea* pollen seasons in some areas of Spain[12,4,27,28,10] and Italy[11], although in some of these areas the pollen season was prolonged to August. Interestingly, the average pollination season in Zagreb and Samobor (Table 2) was of similar duration as in Trieste situated in the north Mediterranean area[11], which can possibly be explained by similar Mediterranean floristic elements, present in both Samobor and Medvednica Mountains[2].

The comparison of total annual chestnut airborne pollen concentration in Zagreb and Samobor, with similar and rare data in other European cities shows that the values recorded in Samobor exceed the figures reported from Vigo, Estepona and Salamanca[10,12]. However, the same data recorded in Zagreb exceed even the figures from Santiago and Ourense, the latter being comparable to Zagreb[4]. The peak pollination season occurs in mid-June in Zagreb and in the first half of July in Samobor, which corresponds to the peak pollination in other European cities.

The pollen counts in the air of the study areas are low, as well as the number of days with more than 50 pollen grains/m$^3$ air, a concentration which is considered critical for susceptible population to develop symptoms associated with the presence of *Castanea* airborne pollen[23]. Although there is a need for information on the allergenic influence of the Fagales taxa in inland Croatia, unfortunately, there hasn't been any published data about any particular Fagaceae allergies of the Croatian population so far. However, accurate information on daily average pollen concentration exceeding 50 *Castanea* pollen grain/m$^3$ air could be of great importance to people suffering from Fagales pollen allergy because of the possibly prolonged period of Fagales pollen season. Our results showed the number of such days for *Castanea* to be considerably low. Evenmore, e.g. in 2004 there was no period in Zagreb with more than 50 pollen grains/m$^3$ air (Table 2). Howerever, it is possible that one of the reasons for the relatively low chestnut pollen concentrations recorded in this study (Table 2) could be the presence of fungus *Cryphonectria parasitica*[8] in Croatia, but to be sure, further complex researches on this problem are necessary.

The correlations between pollen concentration and temperature were positive, except for Samobor in 2004 and 2005 with negative correlations in some cases. However, positive correlations were moderate and significant,

which is also known from some other aerobiological studies[4,11] which suggest that higher temperature conditions are very good for pollination and cause higher pollen concentrations. As the year 2003 was a very warm year (Table 1), we can assume that such high temperatures caused an earlier onset of the pollen season, a higher and earlier daily peak, and also a shorter pollen season (Table 2).

The correlations between pollen concentrations and rainfall were negative, but none were significant, which is also known for Trieste[11]. The absence of significant negative correlation was also noticed earlier in Spain[4,12] and was explained by hourly distribution of rainfall throughout the spring and summer. Thus, we can assume that a similar situation may have occurred in our study areas, although we did not have such precise meteorological data. Furthermore, as suggested in some other aerobiological studies[17–19] the high degree of association between the pollen data from different sampling stations indicates that pollen data from only one station could be sufficient for long-term forecast for similar areas or that such models for one aerobiological station could be based on readings taken from another station. This fact was also proved in our research (Table 5) and showed that some smaller floristic and climate differences between two localities studied, did not cause significant differences for *Castanea* airborne pollen concentrations.

In conclusion, researches of *Castanea* airborne pollen in the air of the cities of Zagreb and Samobor indicated a rule – the shorter the main pollen season, the higher the pollen peak concentration. Generally, *Castanea* has similar pollen seasons in both study areas, which is in accordance with pollen seasons in other areas in Europe. Therefore the pollen season of Fagales pollen is prolonged to summer in the area of inland Croatia, but for a possible prediction and a long-term forecast model for the north-west Croatia, aeropalynological data obtained from only one station are sufficient. Besides allergologists, additional studies and continuous monitoring of chestnut pollen concentrations may prove useful information also for botanists and foresters to follow up the state of chestnut woods, especially those threatened by the fungus *Cryphonectria parasitica*.

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## AEROBIOLOGIJA PITOMOG KESTENA, *CASTANEA SATIVA* MILL., U SJEVEROZAPADNOJ HRVATSKOJ


### S A Ž E T A K

Ciljevi istraživanja bili su analizirati značajke peludnih sezona pitomog kestena (*Castanea sativa* Mill.) i usporediti aerobiološke podatke dviju postaja u sjeverozapadnoj Hrvatskoj. Istraživanje je provedeno u razdoblju od 2003. do 2006. godine tijekom vegetacijske sezone, a za sabiranje uzoraka peludi iz zraka korišten je volumetrijski uređaj za pelud i spore Hirstovog tipa. Sezona pojavljivanja peludi kestena u zraku traje od lipnja do kraja srpnja u oba ispitivana područja što je slično kao i u ostalim europskim gradovima. Uočena je pravilnost – što je kraća peludna sezona to je viša koncentracija peludi. Iako se zbog ljetne polinacije roda *Castanea* može produžiti i sezona pojavnosti simptoma alergijskih reakcija kod osoba osjetljivih na pelud reda *Fagales*, broj dana s količinom peludi većom od 50 u $m^3$ je relativno mali te vjerojatno ne bi značajnije utjecao na alergične osobe. Korelacija koncentracije peluda s temperaturom je pozitivna i signifikantna, a s oborinama je negativna i nije signifikantna. Zbog nesignifikantnih razlika u koncentraciji peludi između dviju aerobioloških postaja, za potencijalne dugoročne prognoze peludnih sezona biljaka reda *Fagales* za sjeverozapadno područje Hrvatske, dovoljni su i aerobiološki podaci dobiveni s jedne postaje.