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Research Article

The influence of meteorological factors on the dynamic of *Ambrosia artemisiifolia* pollen in an invaded area

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Abstract

The aim of the present study was to analyse the effect of weather conditions on *Ambrosia artemisiifolia* air pollen concentrations in the highly invaded area of western Romania. The investigation of *Ambrosia* pollen concentrations was carried out for a period of ten years by means of the volumetric method. *Ambrosia* pollen concentrations had increasing trend over study period. The results of cluster analysis show that two main groups were identified: group A, with lower SPI and group B, with much higher SPI. The statistical correlation between pollen concentrations and meteorological factors was determined by Pearson's test. The relationships between *Ambrosia* pollen concentrations and meteorological parameters, were further assessed using multiple linear regression techniques. The pollen emissions are affected by meteorological factors in the main pollen season. Our results suggest that the abundance of *Ambrosia artemisiifolia* in western Romania is massive. The *Ambrosia* pollen load of Timisoara is most important between 15 August - 15 September. Consequently, this is the most dangerous period of the year for allergic reactions. The investigation of *Ambrosia* pollen behaviour in the atmosphere is a compulsory step for measures to stop the spread and establishing control. *Ambrosia* pollen represents a major health problem and can be considered the main aero allergenic plant pollen in our region.

Keywords: aeroallergen; main pollen season (MPS); seasonal pollen index (SPI)

Introduction

Ambrosia artemisiifolia (short or common, annual ragweed) is an annual monoecious weed and has become a spreading neophyte in Europe. *A. artemisiifolia* is anemophilous, contrary to most Asteraceae. It is an invasive and noxious plant, an important weed in agriculture and source of allergenic pollen (Skjøth *et al*., 2010; Makra *et al*., 2015; Sikoparija *et al*., 2017).

Common ragweed, which is native to North America, can also be found on other continents, including Asia, Australia, Africa, and South America (Essl *et al*., 2015). In European areas most contaminated are Hungary, Romania, Serbia, Croatia, Ukraine, parts of France, northern Italy, but it is also spreading in

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Switzerland, Austria, the Czech Republic, Slovakia, Poland, Germany, Georgia and Bulgaria (Stepalska *et al*., 2008; Smith *et al*., 2013). Ambrosia's pollen is a major aeroallergen and is becoming an extremely serious problem in Europe where is an increasing trend in sensitization of people (Bilińska *et al*., 2017). This pollen is known for its high potential to cause type I allergic reactions in late summer and early autumn (Chen *et al*., 2018). Recent analyses reveal that the ongoing increase in temperature extremes might be contributing to extended seasonal duration and increased pollen load for many aero allergenic pollen taxa in the northern hemisphere (Ziska *et al*., 2019).

The *Ambrosia* genus has only one species, *Ambrosia artemisiifolia,* in the Timisoara region. During the last two decades, an accelerated spread has been observed (Ianovici and Sîrbu, 2007; Ianovici, 2009). In this study, Ambrosia pollen was selected due to its high allergenicity in a four-score scale found in the Romanian pollen index. Allergenicity of *Ambrosia* is the highest indicated by score four (Ianovici, 2007). Accordingly, studying influences of current and past weather conditions on pollen concentrations has of major importance. The aim of this study is to investigate *Ambrosia* pollen presence in western Romania, and to analyze seasonal pollen index, pollen season length, main pollen season, peak days due to the possible relationship between some meteorological parameters and its pollen concentrations.

Materials and Methods

Description of the study site

Timişoara is situated in the western region of Romania (45° 45′ N, 21° 13′ E), at 88 meters above sea level. The climate is temperate continental moderate. The lowest air temperature values are recorded in January and February, with while the maximum ones are reached in July and August. Rainfall is concentrated mainly in the spring and autumn months. The highest relative humidity values were recorded at the beginning of the year, with minimum values being reached in the summer months.

Over the last decades, climatic changes in the region show increasing warm-related temperature extremes (Birsan *et al*., 2019), more frequent of rain showers (Busuioc *et al*., 2016; Manea *et al*., 2016), decreasing snow depth and wind speed (Birsan *et al*., 2013, 2020) – influencing the human health (Dobrinescu *et al*., 2015), terrestrial ecosystems (Mihai *et al*., 2018a,b) or hydrological regime (Birsan, 2015).

Mean values of the meteorological data provided by Meteo Romania (the National Meteorological Administration) are shown in Table 1.

Sampling design

The aerobiological measurement was performed using volumetric pollen trap of the Hirst design (Hirst, 1952). The air sampler is located on top of the building of the West University, approximately 20 m above the ground surface. Pollen grains were sampled continuously. The sampling airflow rate was 10 L/min. The transparent tape used for catching air pollen was replaced every week and cut into segments corresponding to 24 h periods. These segments were mounted on slides with glycerin and basic fuchsin under coverslips. Pollen grains were counted along four longitudinal transects (using a light microscope at 400× magnification) (Cariñanos *et al*., 2000; Ianovici, 2015). Daily pollen concentrations are expressed as particles per cubic metre of ambient air (P/m^3). Because of some irrelevant circumstances, the monitoring was interrupted in 2005.

Qualitative analysis

The Seasonal Pollen Index (SPI), the sum of the daily pollen concentrations in a given season, is expressed as the number of pollen grains (Comtois, 1998). We apply the cumulative sum method to calculate the main pollen season (MPS). The start of the season was defined as the date when 5% of the pollen count was trapped and the end of the season as the date when 95% of the pollen count was reached (Nilsson and Persson,1981).

In order to evaluate the impact on human health, several research studies report different critical threshold values of pollen concentrations that provoke the first symptoms in sensitized patients. In this study, concentrations above 20 P/m^3 were defined as high (Makra *et al*., 2005; Stępalska *et al*., 2016).

Table 1. Meteorological conditions in Timisoara (2000-2010)

		Mean air temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Daily precipitati on (mm)	Relative humidity (%)	Sunshine hours (h)	Atmospher ic pressure (mb)	Mean wind speed (m/s)	Mean daily maximum wind speed (m/s)
2000	FY	12.45	19.22	6.95	0.81	65.98	6.89	1006.14	1.81	3.14
	SSD	20.50	28.07	14.06	0.65	57.26	8.74	1003.82	1.92	3.17
	PD	20.49	28.03	14.06	0.65	57.11	8.77	1003.89	1.93	3.18
	MPS	21.01	29.00	14.26	0.46	56.12	8.63	1004.31	1.71	2.88
2001	FY	11.37	17.23	6.65	1.88	73.84	5.95	1005.28	1.98	2.97
	SSD	19.46	26.44	14.22	2.20	72.08	7.84	1004.09	1.77	2.75
	PD	19.58	26.51	14.33	2.22	71.89	7.87	1004.00	1.78	2.76
	MPS	18.96	25.87	13.77	3.54	71.36	7.40	1003.12	1.86	2.74
2002	FY	12.27	18.22	7.44	1.56	69.79	5.96	1005.86	1.91	2.85
	SSD	18.90	25.38	14.04	2.37	72.00	6.87	1003.12	1.58	2.45
	PD	19.02	25.53	14.13	2.41	71.86	6.89	1003.15	1.58	2.46
	MPS	18.85	25.41	14.13	2.36	71.99	6.44	1003.15	1.65	2.49
2003	FY	11.05	17.36	5.83	1.58	69.31	6.62	1007.19	1.87	2.76
	SSD	21.01	28.57	14.61	1.40	59.18	8.98	1005.72	1.76	2.67
	PD	21.15	28.69	14.71	1.40	58.96	9.02	1005.56	1.77	2.69
	MPS	20.83	28.43	14.08	1.48	53.37	8.75	1005.61	1.70	2.67
2004	FY	11.12	16.85	6.50	1.93	71.61	5.78	1005.94	1.95	2.88
	SSD	19.20	26.45	13.38	1.98	65.65	8.40	1005.76	1.81	2.69
	PD	19.32	26.59	13.43	1.86	65.45	8.49	1005.75	1.80	2.67
	MPS	19.86	27.85	13.72	1.74	65.27	9.51	1005.94	1.71	2.73
2006	FY	11.18	17.19	6.49	1.59	72.72	5.46	1007.09	1.89	2.75
	SSD	19.14	26.50	13.69	1.91	69.35	7.04	1004.73	1.73	2.61
	PD	19.25	26.58	13.79	1.91	69.00	7.06	1004.65	1.76	2.65
	MPS	18.44	25.80	12.95	1.21	66.97	7.11	1006.52	1.83	2.80
2007	FY	12.45	18.58	7.40	1.78	68.18	5.66	1005.81	1.98	2.88
	SSD	20.96	28.58	14.84	1.87	61.15	7.57	1003.66	2.00	2.95
	PD	21.10	28.69	14.96	1.87	60.92	7.61	1003.54	2.02	2.98
	MPS	20.51	27.51	15.42	1.72	64.89	6.82	1003.01	1.90	2.93
2008	FY	12.23	18.53	7.26	1.61	69.28	5.55	1006.01	1.92	2.81
	SSD	20.11	27.33	14.11	1.65	63.32	7.46	1004.02	1.73	2.52
	PD	20.22	27.41	14.24	1.65	63.30	7.49	1004.02	1.74	2.53
	MPS	21.50	30.06	14.86	0.92	58.26	8.39	1003.38	1.72	2.71
2009	FY	12.31	18.45	7.24	1.71	73.96	5.50	1004.28	1.84	2.62
	SSD	21.07	29.01	14.50	0.76	67.81	8.10	1005.79	1.63	2.34
	PD	21.13	29.10	14.55	0.76	67.73	8.14	1005.77	1.63	2.34
	MPS	21.64	29.67	15.10	0.33	63.54	8.39	1006.47	1.64	2.31
2010	FY	11.66	17.37	7.13	2.17	79.99	4.88	1002.99	1.92	2.72
	SSD	21.00	27.89	15.58	1.69	74.40	7.19	1003.69	1.75	2.47
	PD	20.97	27.83	15.54	1.57	74.47	7.18	1003.80	1.75	2.45
	MPS	20.33	27.25	14.77	2.47	72.63	7.32	1004.37	1.79	2.60

Statistical procedures

To test assumptions of ANOVA data were tested for normality of variances with Shapiro-Wilk's test. The similarities between pollen season parameters were determined on the basis of the results of cluster analysis. Data clustering was performed using the Ward's method and the k-means method. In this way a pollen season is classified into a particular cluster by minimizing within the cluster variance and maximizing between the cluster variances (Piotrowska and Kubik-Komar, 2012). Pollen seasons were grouped in terms of the season parameters (seasonal pollen index – SPI; pollen season length; main pollen season – MPS; number of days with pollen concentrations over $20P/m^3$; concentration in the peak day of the year).

The statistical techniques usually employed to study pollen concentrations trends are correlation and regression analyses (García-Mozo *et al*., 2014; Ianovici, 2017). Simple linear regression of SPI against to year was carried out for *Ambrosia* pollen. After that, the correlation coefficients between daily air pollen concentrations and daily meteorological variables were analysed. The Pearson's parametric test was chosen because daily pollen concentrations are normally distributed. Pearson's correlation test was performed in order to identify the meteorological parameters that are likely to influence the annual dynamic of the *Ambrosia* airborne pollen. The significance was calculated for $P<0.05$. We analysed the effect of the main meteorological variables on pollen concentrations taking the pollen season length as a whole, over a full year (FY). The meteorological variables considered in this study were: mean, maximum and minimum air temperature (°C); relative humidity (%); atmospheric pressure (mb); number of sunshine hours (h); mean daily precipitation (mm), maximum daily wind speed (m/s); mean wind speed (m/s). In the next step, the daily values of the current (meteorological parameters from the same sampling day -SSD) and preceding (meteorological parameters from the previous day – PD) weather variables influencing the pollen season length were used in this study. Finally, the values included in the main pollen season (MPS) were used for the statistical analysis.

The accumulated heat is introduced in some models that forecast MPS particularities (Zhang *et al*., 2015; Ritenberga *et al*., 2018). The impact of cumulative temperature (average, maximum and minimum temperature) and cumulative sunshine hours calculated from 1 January until the date of the beginning of the pollen season was examined. This method did not give positive results in our case.

Complementary with the Pearson's correlation test, the multiple regression analysis was used in order to determine how much of total variance in these pollen concentrations can be explained by meteorological variables.

The all data were processed with Microsoft Excel for Windows, PAST and SPSS software package.

Results

In the first part of this section, there will be presented the results of aerobiological observations based on airborne pollen concentrations, and the second part shows the meteorological aspects of this study.

The aerobiological parameters in each year (excluding 2005 because of incomplete sampling) are given in Table 2.

Table 2. Aerobiological parameters of *Ambrosia* pollen concentrations in the particular clusters

Cluster	Year	SPI (Seasonal Pollen Index)	Number of days with pollen concentrations higher than $20P/m^3$	Pollen season length		MPS- days (90% method)		Seasonal maximum daily count (concentration in a peak day)
A	2000	2848	42	116	20.06-13.09	41	10.08-19.09	170
	2001	2329	40	96	11.07-14.10	47	06.08-21.09	95
	2002	4473	59	98	08.07-01.10	69	25.07-13.10	234
	2003	3603	48	89	02.07-28.09	43	06.08-17.09	319
	2004	3131	38	110	24.06-11.10	44	03.08-15.09	220
	2007	3367	36	84	03.07-24.09	28	15.08-11.09	230
B	2006	6899	45	89	18.07-14.10	30	19.08-17.09	468
	2008	5217	51	109	20.06-06.10	38	08.08-14.09	320
	2009	5591	54	100	04.07-11.10	39	09.08-16.09	292
	2010	5942	47	92	27.07-26.09	35	10.08-26.09	413

In the years examined in Timisoara, the number of days when the threshold value was exceeded was high. The highest value was seen for 59 days in 2002. The concentration in a peak day showed great variation between years and varied 95-468 P/m^3. The peak daily concentration was noted on September 6, 2006. The seasonal pollen index of Ambrosia pollen ranged 2329-6899 pollen grains. The mean seasonal pollen index obtained during the studied period (2000-2010) was 4340 P/m^3. The lowest seasonal pollen index was recorded in 2000 and the highest annual sum in 2006. The pollen season length lasted in Timisoara from 116 days in 2000 to 84 days in 2007. Among the years studied, 2007 was characterized by a short main pollen season. Based on the available data set, it was established that the longest main pollen season was in 2002. Start dates were found in the first half of August (9 years) or in the second half of August (1 year - 2006). Usually small amounts of airborne pollen are present at the start of the seasons (at the end of June and July) (Figure 1).

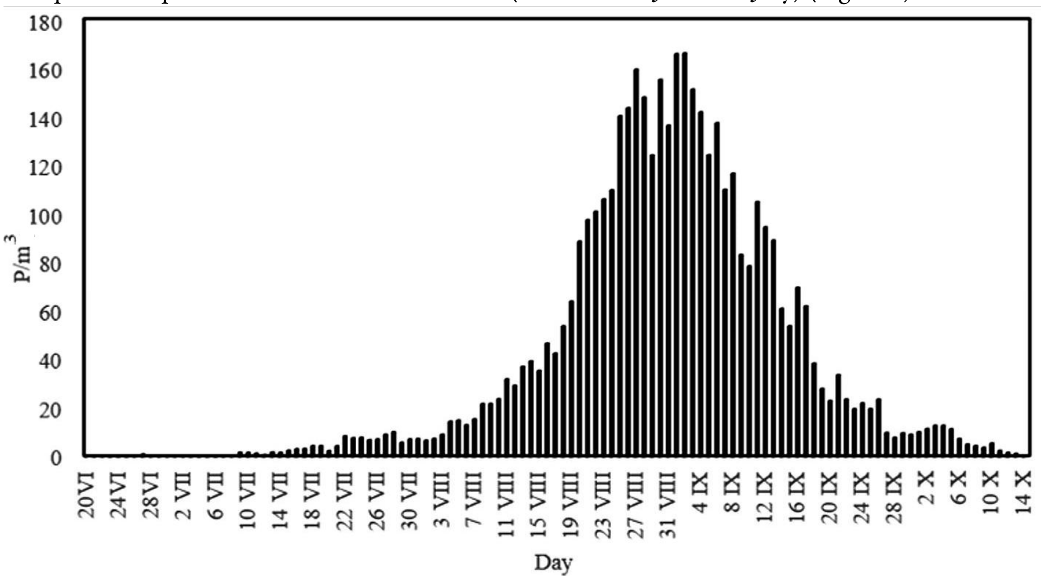

Figure 1. Mean daily *Ambrosia* pollen concentrations over the pollen season (20 June – 14 October) in Timisoara from 2000 to 2010

The one-way ANOVA test revealed that the Ambrosia pollen concentrations do differ among years ($F=4.148$; $p > 0.00002447$). More significantly statistically differences are in the case of taking into account only the days with the presence of pollen in the ambient air ($F=7.049$; $p > 0.0000000006382$).

The similarity between atmospheric pollen concentrations was determined using cluster analysis. The results are presented in the form of dendrogram (Figure 2). In terms of pollen season parameters, two clusters were distinguished: 2000, 2001, 2002, 2003, 2004 and 2007 (A); 2006, 2008, 2009, and 2010 (B). The 2006 season was characterized by a late onset, a short duration, and at the same time, very abundant pollen emission. Moreover, apart from the year 2006, a high SPI were observed in 2008, 2009 and 2010.Temporal linear trend show the Ambrosia pollen concentrations increases at a steady rate during the 10 years of study (Figure 3).

In the second part, the meteorological aspects will be presented. Assessment of the influences of meteorological parameters on pollen concentrations in the atmosphere, based on systemized results, is presented in Table 3.

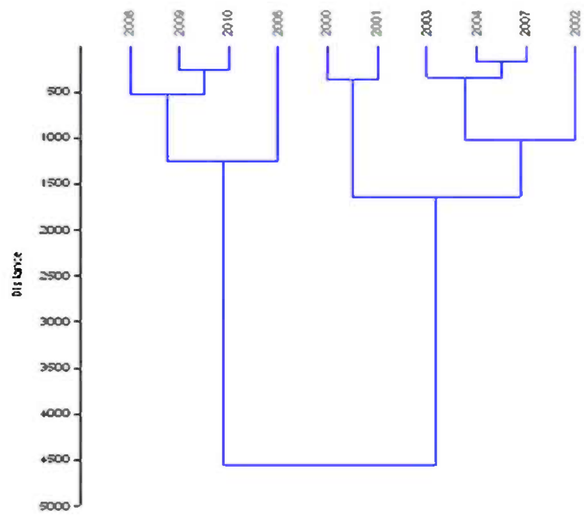

Figure 2. Groups of *Ambrosia* pollen seasons identified on the basis of the seasonal parameters in Timisoara in the period 2000-2010

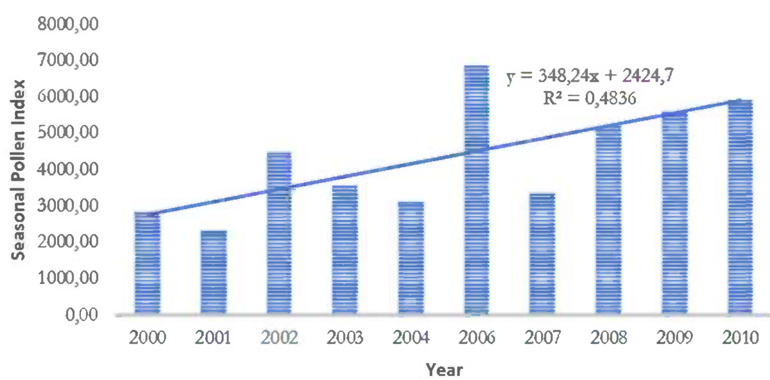

Figure 3. Linear regression of the Seasonal Pollen Index

The correlations between the Ambrosia pollen and meteorological factors over a full year (FY)

The correlation coefficients were not statistically significant in the case of all the meteorological factors in question. All the pollen concentrations increase when the temperatures (mean, maximum, minimum) increases. The strongest correlation was seen for 2001 and 2009. Pearson's correlation analysis also revealed the negative and significant influence of relative air humidity (with average annual values over 65%) on airborne pollen. The total daily hours of sunshine had obtained positive and significant correlations with pollen concentrations in all years (except 2000). The correlation coefficients were lower for daily average wind speed, daily maximum wind speed and atmospheric pressure. In relation to daily average wind speed, the correlation was significant and negative in the case of 2002, 2003, 2008 and 2009. Weak negative correlations were observed in the case of quantities of precipitations. The atmospheric pressure influences are mixed between 2000-2010.

Table 3. Pearson's correlation coefficients between *Ambrosia* pollen concentrations and main meteorological parameters

Pearson correlation		Mean daily average temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Quantities of precipitations (L/m^2)	Daily average relative humidity (%)	Sunshine hours (h)	Atmospheric pressure (milibars)	Daily average wind speed (m/s)	Daily max. wind speed (m/s)
2000	FY	**.293****	**.290****	**.303****	-.024	**-.191****	.064	**-.120***	-.007	-.023
	SD	-.044	-.009	-.017	.001	.088	-.181	-.031	-.080	-.071
	PD	.010	.050	.024	-.059	.084	-.141	-.022	-.091	-.110
	MPS	.095	.138	.043	-.086	-.085	-.086	.001	-.160	-.137
2001	FY	**.339****	**.340****	**.348****	.061	-.098	**.158****	**-.108***	-.092	-.091
	SD	-.031	.007	-.065	.073	-.115	.002	**-.219***	-.025	-.097
	PD	-.030	.010	-.077	.006	-.153	.013	-.136	-.035	-.098
	MPS	.129	.171	.083	-.102	-.196	.155	-.164	-.226	-.203
2002	FY	**.284****	**.299****	**.286****	-.019	-.026	**.162****	-.039	**-.128***	**-.144****
	SD	.003	.133	-.105	-.176	**-.278****	**.233***	**.395****	-.011	-.122
	PD	-.009	.112	-.101	-.132	**-.268****	**.203***	**.352****	-.059	-.153
	MPS	.175	**.306***	.002	-.199	**-.422****	**.403****	**.388****	-.057	-.199
2003	FY	**.278****	**.301****	**.247****	-.077	**-.302****	**.195****	-.070	**-.143****	-.092
	SD	-.060	.084	**-.233***	-.130	**-.274****	.110	-.034	**-.286****	-.191
	PD	-.095	-.014	**-.226***	-.024	**-.269***	.045	.005	**-.253***	-.181
	MPS	-.001	.157	-.171	-.173	-.107	.210	-.199	**-.323***	-.221
2004	FY	**.260****	**.282****	**.239****	-.073	**-.212****	**.184****	**.136****	-.054	.005
	SD	-.154	-.063	**-.236***	-.139	-.110	-.001	**.436****	-.015	.144
	PD	-.142	-.046	**-.229***	-.143	-.051	-.004	**.418****	-.042	.104
	MPS	**-.574****	**-.530****	**-.594****	-.195	**-.438****	-.263	**.739****	.157	.257
2006	FY	**.219****	**.229****	**.221****	.025	-.100	**.121***	-.034	-.033	.003
	SD	**-.258***	**-.230***	**-.262***	.002	.005	.004	.199	.063	.105
	PD	**-.245***	**-.240***	**-.249***	-.018	.035	-.043	.199	.038	.105
	MPS	**-.508****	**-.369***	**-.464****	.250	**.395***	.033	.116	-.122	-.036
2007	FY	**.301****	**.288****	**.335****	-.041	**-.136****	**.129***	-.071	.020	.071
	SD	.048	.016	.155	-.093	.040	-.029	.048	.036	.151
	PD	.091	.061	.202	-.098	.036	.035	.068	-.140	-.009
	MPS	**.428***	**.409***	**.434***	-.195	**-.506****	.285	.204	**.425***	**.533****
2008	FY	**.309****	**.319****	**.303****	-.006	**-.225****	**.170****	-.053	**-.119***	-.082
	SD	.008	.082	-.010	-.015	-.113	.019	.116	-.112	-.015
	PD	-.002	.103	-.080	-.068	-.144	.068	.150	-.147	-.019
	MPS	-.259	-.242	-.193	.208	.218	-.154	**.520****	-.270	-.228
2009	FY	**.351****	**.368****	**.343****	-.084	**-.246****	**.255****	**.105***	**-.105***	-.099
	SD	.121	**.215***	.135	-.079	**-.350****	.093	.118	-.041	-.048
	PD	.130	**.220***	.090	-.079	**-.400****	.168	**.224***	-.082	-.099
	MPS	.233	**.412****	.148	.289	**-.358***	.186	-.131	-.080	-.014
2010	FY	**.266****	**.272****	**.249****	-.031	**-.216****	**.190****	.048	-.065	-.047
	SD	**-.361****	**-.269****	**-.449****	.004	-.137	-.007	.068	-.010	.048
	PD	**-.311****	**-.260***	**-.352****	.069	**-.210***	.017	.080	.037	.068
	MPS	**-.455****	-.294	**-.585****	-.187	-.106	-.037	-.107	-.083	-.079

Statistically-significant correlations at 0.01 and 0.05 levels are marked by "**" and "*", respectively.

The correlations between the Ambrosia pollen and meteorological factors only from the same sampling day (SSD)

Secondly, we took into consideration all days when the pollen grains were present in the air. Overall, the correlations with temperature were not significant when data is restricted to the period of pollen grains being released. However, significant negative correlations were observed in the case of temperatures in 2006 and 2010. The results have shown that the level of *Ambrosia* pollen in 2002 was significant positive affected by sunshine hours on the same day. In relation to daily average wind speed, the correlation was significant and negative only in the case of 2003. For three years (2002, 2003, 2009) was negative correlation detected between pollen concentrations and relative air humidity. The effect of atmospheric pressure on airborne pollen concentrations can be positive (2002 and 2004) and negative (2001).

The correlations between the Ambrosia pollen and meteorological factors from the previous day (PD)

We have chosen the weather variables on the day before to the appearance of airborne pollen to see if it confirms the statistical data on the SSD. Weak positive correlation was observed in the case of maximum temperature in a day earlier for 2009. In some years (2003, 2004, 2006, 2010) *Ambrosia* pollen presented a significant negative correlation with minimum temperature on the previous day. Pollen concentrations showed a negative and significant correlations with daily average relative humidity on the day before sampling (2002, 2003, 2009 and 2010). The relationship with the sunshine hours was not so close. The effect of atmospheric pressure on airborne pollen concentrations was positive (2002, 2004 and 2009).

The correlations between the Ambrosia pollen and meteorological factors from the main pollen season (MPS)

Over a full year, the pollen season length exhibits a strong temporal course, with a winter, spring and late autumn periods free of pollen. The MPS is applied to reduce the coefficient of variability of pollen concentration data. Overall, the negative correlations with relative humidity were significant when data is restricted to the MPS. The same meteorological variable (mean temperature, maximum temperature, minimum temperature or wind speed) may have different influences in different years.

Ambrosia airborne pollen dependence on the weather in multiple linear regression analysis

Table 4 shows the results of multiple linear regressions. Only the values of the statistically significant coefficients were selected. The most important variables statistically significantly different to 0 were not the same for each year. Unstandardized coefficients B indicate how much the Ambrosia pollen concentration (dependent variable) varies with an independent variable when all other independent variables are held constant. Unstandardized coefficients B for the same variable were negative in some cases and positive in others (sunshine hours, atmospheric pressure, wind speed). The most surprising aspects are caught in the case of temperature. Lowering the temperature from very high values and raising the temperature from lower values are associated with increasing pollen concentrations. For Ambrosia pollen concentrations, meteorological parameters explained 7.5% of the total variance in 2006 and 18.3% in 2009. The values of the R^2 obtained over a full year (FY) were quite inferior to those of SSD, PD or MPS. The proportion of variance explained during MPS is high (ranged from 13.70% until 68.40%).

Discussion

This study highlights the difficulty in interpretations relating the associations between Ambrosia pollen concentrations and meteorological variables. Airborne pollen concentrations over the full year (FY) were found to be positively correlated with several climatic factors (mean temperature, maximum temperature, minimum temperature) and negatively correlated with others (quantities of precipitations, daily average wind speed). In this way, we included the data from all phenological phases of plants. The results of Pearson's correlation test showed that the atmospheric pollen release is occasionally affected by the daily maximum wind speed. In the present study, we found a positive correlation between Ambrosia pollen concentrations and sunshine hours. Because photoperiod is defined as the period of sunshine hours necessary for flowering, it can be considered that sunshine hours are correlated directly with the specific photoperiod of this plant (Vázquez *et al*., 2003). The level of daily pollen concentration is also affected by the prior meteorological conditions (Matyasovszky *et al*., 2015; Wozniak and Steiner, 2017). When data is restricted to the period of pollen grains being released, temperature (with all its components) was the most correlated with the pollen concentrations. Relative humidity and sunshine hours also showed to have some correlations on the pollen emission. Nevertheless, the results for each year were different depending on the analyzed period (FY, SSD, PD and MPS).

Table 4. Multiple linear regression variable results

Year		Multiple correlation coefficient (R)	Coefficient of determination (R Square)	ANOVA - F	Proportion of variance explained	Explanatory variables	Unstandardized Coefficients B	*P*-value (Sig.)
2000	FY	0.359	.128	5.821	12.86%	sunshine hours	-1.364	.00205
	SD	0.29	.084	1.084	8.40%	sunshine hours	-2.787	.0391
	PD	0.328	.108	1.422	10.8%	sunshine hours	-2.917	.02837
	MPS	0.37	.137	1.866	13.70%	sunshine hours	-4.012	.0034
2001	FY	0.393	.155	7.241	15.50%	maximum temperature	1.107	.0365
						minimum temperature	1.361	.04049
	SD	0.453	.205	2.470	20.50%	daily average relative humidity	-1.375	.0094
						atmospheric pressure	-1.885	.0089
	PD	0.429	.184	2.154	18.40%	daily average relative humidity	-1.667	.0020
	MPS	0.498	.248	1.356	24.80%	atmospheric pressure	-2.759	.0324
2002	FY	0.421	.177	8.510	17.7%	mean daily average temperature	-5.598	.0066
						maximum temperature	4.312	.0001
						minimum temperature	2.997	.0171
						daily average relative humidity	.378	.0484
						atmospheric pressure	.728	.0238
						daily max. wind speed	-5.158	.0354
	SD	0.586	.343	5.112	34.30%	maximum temperature	11.968	.0009
						atmospheric pressure	3.982	.0013
						daily average wind speed	34.066	.0028
						daily max. wind speed	-19.838	.0046
	PD	0.534	.286	3.908	28.60%	mean daily average temperature	-14.757	.046
						maximum temperature	10.701	.0040
						atmospheric pressure	3.319	.0085
						daily average wind speed	23.933	.0416
						daily max. wind speed	-17.291	.0178
	MPS	0.617	.381	4.031	38.10%	maximum temperature	10.367	.0317
						atmospheric pressure	3.503	.0320
						daily average wind speed	32.500	.0291
						daily max. wind speed	-25.012	.0104
2003	FY	0.418	.174	8.335	17.40%	maximum temperature	2.205	.04994
						daily average relative humidity	-.841	.0001
						sunshine hours	-1.408	.0287
						daily average wind speed	-12.396	.0044
	SD	0.603	.364	5.016	36.40%	daily average relative humidity	-2.368	.0012
						atmospheric pressure	-3.349	.0211
						daily average wind speed	-46.188	.0069
	PD	0.566	.320	4.140	32%	daily average relative humidity	-3.013	.00006
						daily average wind speed	-42.387	.01562
	MPS	0.696	.485	3.453	49.50%	sunshine hours	11.753	.0174
						atmospheric pressure	-10.782	.0006
2004	FY	0.377	.142	6.552	14.20%	maximum temperature	2.500	.0051
						atmospheric pressure	.829	.0002
	SD	0.555	.308	4.945	30.80%	atmospheric pressure	3.684	.00015
						daily max. wind speed	19.141	.0029
	PD	0.514	.264	3.981	26.40%	atmospheric pressure	3.691	.00022
						daily max. wind speed	17.057	.00962
	MPS	0.827	.684	8.166	68.40%	atmospheric pressure	5.435	.0030
2006	FY	0.274	.075	3.207	7.50%	mean daily average temperature	-8.800	.0477
						maximum temperature	5.171	.0207
	SD	0.402	.161	1.689	16.10%	-	-	-
	PD	0.386	.149	1.533	14.90%	-	-	-
	MPS	0.666	.443	1.770	44.30%	-	-	-
2007	FY	0.413	.171		17.10%	mean daily average temperature	-5.638	.01074
						maximum temperature	2.313	.04719
						minimum temperature	5.268	.00009
						daily average wind speed	-9.432	.02913
						daily max. wind speed	8.039	.00465
	SD	0.445	.198	2.026	19.80%	minimum temperature	13.660	.01641
	PD	0.532	.283	3.251	28.30%	minimum temperature	18.340	.00086
						daily average wind speed	-36.470	.03303
	MPS	0.759	.575	2.710	57.50%	-	-	-
2008	FY	0.395	.156	7.324	15.60%	mean daily average temperature	-5.760	.03778
						maximum temperature	3.662	.01090
						minimum temperature	3.812	.03468
						daily average relative humidity	-1.115	.00149
						sunshine hours	-2.543	.02333
						daily average wind speed	-13.018	.02982

	SD	0.419	.176	2.344	17.60%	mean daily average temperature	-21.768	.02990
						maximum temperature	11.678	.00687
						daily average relative humidity	-3.237	.00809
	PD	0.504	.254	3.748	25.40%	mean daily average temperature	-21.828	.02199
						maximum temperature	14.277	.00060
						daily average relative humidity	-3.324	.0045
	MPS	0.715	.511	3.253	51.10%	atmospheric pressure	15.118	.0007
2009	FY	0.428	.183	8.836	18.30%	mean daily average temperature	-8.314	.0017
						maximum temperature	4.323	.0023
						minimum temperature	5.523	.0015
						daily average relative humidity	-.601	.0175
	SD	0.624	.389	6.369	38.90%	mean daily average temperature	-45.866	.000001
						maximum temperature	19.603	.000189
						minimum temperature	30.395	.000009
						daily average relative humidity	-5.190	.0000002
	PD	0.601	.361	5.654	36.10%	mean daily average temperature	-35.520	.0001
						maximum temperature	14.532	.0063
						minimum temperature	25.585	.0002
						daily average relative humidity	-4.804	.000002
	MPS	0.719	.517	3.453	51.70%	mean daily average temperature	-53.085	.0076
						maximum temperature	36.997	.0024
						quantities of precipitations	18.906	.0245
						daily average relative humidity	-3.484	.0415
2010	FY	0.291	.085	3.659	8.50%	-	-	-
	SD	0.59	.348	4.862	34.80%	daily average relative humidity	-5.959	.0013
	PD	0.615	.378	5.539	37.80%	mean daily average temperature	-34.143	.0188
						daily average relative humidity	-9.453	.0000006
	MPS	0.748	.560	3.535	56%	-	-	-

The Ambrosia MPS exhibits a distinct pattern. Ambrosia is most commonly a short-day plant that begins flowering when days begin to shorten (Ziska *et al*., 2011; Lo *et al*., 2019). The pollen season presents similar behaviour and duration in Hungary, Serbia and Croatia, which can be explained by the biogeographical and bioclimatic conditions. Some researchers have shown the significant and positive correlations of daily pollen concentrations with daily mean temperature (Bartkova-Scevkova, 2003; Štefanić *et al*., 2005; Peternel *et al*., 2006), daily maximum temperature (Stepalska *et al*., 2008), daily mean wind speed (Kasprzyk, 2008), and daily maximum wind speed (Puc, 2006). They were also mentioned the significant and negative correlations with relative humidity (Kasprzyk, 2008) and rainfall (Peternel *et al*., 2005; Peternel *et al*., 2006,). Ambrosia pollen concentrations were negatively correlated with rainfall in several studies (Barnes *et al*., 2001), while in others, no statistically significant correlations were found (Bartkova-Scevkova, 2003). It was established that the relation between higher temperatures and higher pollen concentrations is stronger in urban than in rural locations (Ziska *et al*., 2003). Aerobiological monitoring indicates that after release, air pollen can be transported on the order of 10-1000 km (Wozniak and Steiner, 2017). Long-range pollen transport may have an important effect on local pollen concentrations (Kaplan *et al.,* 2003; Makra *et al*., 2010; Šaulienė and Veriankaitė, 2012; Celenk and Malyer, 2017). As an example, for Szeged, Hungary, annual pollen amount transported by the atmospheric circulation is 27.8% of the annual total pollen. From this quantity, 7.5% is added to (due to transport), while 20.3% is subtracted from (e.g. because of wash-out by frontal rainfalls going towards Szeged) local sources (Makra *et al*., 2016).

Statistical methods used in this research were complementary, since they described the various influences of meteorological factors on the dynamic of pollen concentrations. We can consider that the correlations identified as statistically significant are not consistent enough. In this study, multiple regression analysis was found to be a valuable technique for exploring and identifying the meteorological parameters closely associated with Ambrosia pollen concentrations (Ianovici *et al.*, 2015). For Ambrosia, the multiple linear regressions with statistically valid parameters varied with the period of presence of pollen (FY, SSD, PD, MPS) and the analysed year. The air temperature was the meteorological variable that influenced the pollen concentrations the most. Relative humidity also showed to have some effect on the dynamics of pollen concentrations present in ambient air. The values of the coefficients of determination obtained during the 2000-2010 period indicates that the dynamic of these pollen concentrations can be associated to other factors such as the different stages of vegetative development of plants. Obviously, pollen release and emission depend on the dehiscence of the male flowers. Anther dehiscence is sensitive to abiotic stress in the environment (Franchi *et al.*, 2007). The stomium opening of anther seems to be a programmed event but the wall outward bending is much more dependent on the environment (Carrizo García *et al.*, 2006). Former studies on excised floral organs of Ambrosia showed that the opening of flowers is controlled by the relative humidity and temperature (Bianchi *et al.*, 1959). Positive significant relationship with daily maximum temperature may be explained by anthers dehiscence and pollen grains release when walls of anthers are dehydrated. This dehydration process is facilitated by the maximum temperatures (Makra *et al.*, 2012).

When the relative humidity is favourable, a gradual increase in the daily mean temperature can intensify vegetative and generative functions, which can lead to increased pollen concentrations (Gioulekas *et al.*, 2004). When the relative humidity values are low, excessive increases in average temperature values may slow down or inhibit pollination. Under these conditions, the plants invest resources in limiting water losses, to the detriment of the generative functions. This may be the explanation for which the daily mean temperature showed inverse association with Ambrosia pollen concentrations (Makra *et al.*, 2012).

In our study, relative humidity was inversely associated with the Ambrosia pollen concentrations. It is accepted that the pollen release and emission is associated with shrinkage and rupture of anthers at low relative humidity. On the other hand, the higher relative humidity may stop or delays the opening of pollen sacks (Bianchi *et al.*, 1959). More than that, humid air makes pollen grains stick together, which contributes to this inverse association (Kozlowski and Pallardy, 2002; Gioulekas *et al.*, 2004).

In this study, the role of quantities of precipitations in *Ambrosia* pollen concentrations is not clear. Rainfall is not associated with daily pollen concentrations (except MPS for 2009). It is known that rainfall is followed by a temperature drop. Some researchers consider that the involvement of heavy rainfall is complex and could decrease pollen concentrations (Fornaciari *et al.*, 1992; Galan *et al.*, 2000; Hernandez-Ceballos *et al.*, 2011; Makra *et al.*, 2012).

It is obvious that the release of allergenic pollen of Ambrosia depends both on weather variables and phyto-physiological status of plants. Generally, time dependent daily pollen concentrations are influenced by numerous underlying processes: genetic attributes of taxa, soil type and location specific nutrient availability, land use changes, the characteristics of the root zone, resuspension or disruption of the pollen grains, long-range pollen transport, pollen grains as condensation nuclei etc (Matyasovszky *et al.*, 2015). By several orders of magnitude more pollen grains were found at ground level when *Ambrosia* pollen were collected over an experimental created source (Šikoparija *et al.*, 2018). Also, air pollution and climate change could influence pollen morphology and could increase the aggressiveness of allergens (Ianovici *et al.*, 2008; Zhao *et al.*, 2016). *Ambrosia* plants have been found to produce more pollen as the CO2 levels increase (Ziska and Caulfield 2000; Ziska *et al.*, 2003; Singer *et al.*, 2005). However, the level of pollen concentrations and the pattern of pollen seasons, can also be influenced by human activity (Kasprzyk and Walanus, 2010).

Ambrosia artemisiifolia is a highly prevalent taxon in the Western Plain (which is part of the Pannonian Plain) (Juhász *et al.*, 2004; Zink *et al.*, 2011). *Ambrosia* in western region of Romania discharges more pollen grains into the atmosphere than all other allergenic plants (Ianovici and Sîrbu, 2007). For example, *Ambrosia* was the best-represented non-arboreal pollen type throughout the 2009 study period (an average of 19.83% of the annual total) (Ianovici *et al*., 2013a). At the same time, evaluations of monosensitization patients (51.44%) were showed that *Ambrosia* pollen is principal outdoor aeroallergen (Ianovici *et al*., 2013a). The recent data confirm that Ambrosia pollen is an important allergen for the Southeast region of Romania (Popescu and Tudose, 2011; Leru *et al*., 2019). Moreover, *Ambrosia* pollen was the most important aeroallergen in all of the urban areas sampled during August 2008 (Timisoara, Cluj-Napoca, Bucuresti, Brasov) (Ianovici *et al*., 2013b). In these big cities of Romania, the number of days exceeding the threshold value 20 g/m^3 (Jäger, 1998) was high: 29 for Timişoara, 25 for Bucharest, 19 for Cluj-Napoca, and 12 for Brasov. In Timişoara and Bucharest, the probability of becoming exposed to high concentrations of Ambrosia pollen and airborne fungal spores (*Cladosporium* and *Alternaria*) at the same time is high in August. Anthesis of flowers is prolonged till the beginning of October, along with that of plants belonging to other taxa such as Poaceae, Artemisia and Chenopodiaceae/Amaranthaceae. Furthermore, Ambrosia's pollen can play a role together with Artemisia's pollen on allergic respiratory symptoms, which seen in August-September period (Biçakçi and Tosunoğlu, 2015).

A. artemisiifolia produces very large amounts of pollen and even small populations can be responsible for an increased pollen exposure. Analysis of the data indicated that Ambrosia pollen concentrations in Timisoara vary from year to year. In 1999, the atmosphere did not appear to be biologically polluted because of the pollen produced by *A. artemisiifolia*. The preliminary study for that year indicated a SPI with 722 pollen grains (Faur *et al*., 2001). In cluster B which was identified in the present study (covering the years 2006, 2008, 2009, 2010), the average SPI was 5912 pollen grains. Time series analysis can reveal trend estimation in our data observed and predict future trends. In our opinion, the rising trend of the seasonal pollen index reflects the expansion of Ambrosia populations. It is important to emphasize that stable populations are identified in all parts of Romania (Ianovici, 2011).

August and September were the months with the highest daily *Ambrosia* pollen concentrations during the study period. This fact confirms the results received by Makra *et al*. (2004) and Makra *et al*. (2005). They received that, based on two analyses, the most extreme ragweed pollen load at Szeged, Hungary occurs between August 16 - September 13 (Makra *et al*., 2014) or between August 20 - September 11 (Makra *et al*., 2005). This comparison confirms our result with the physical background that the two cities, Timisoara, Romania and Szeged, Hungary are located only 120 km from each other, both are found in the Pannonian Plain and have the same climate, which is of Köppen Cf climate zone (temperate-warm climate with an almost even distribution of precipitation; Köppen, 1931), or Trewartha's D.1 climate zone (continental climate with long warm season; Trewartha, 1943).

This highly successful pioneer species is adapted to low nutrient environments. The species is both a weed colonizing crops and a ruderal plant developing in open disturbed habitats (such as roadsides, or riverbanks) (Fumanal, 2006). They occur in stubble fields, especially in abandoned places around settlements. The change in land use in Romania was important from the year 1990 until to the year 2000. In this time many fields were abandoned (Ianovici, 2017). Subsequent, the main *Ambrosia* infested areas were the construction sites, the new residential places with deficient infrastructure and some cultivated land. In the specialized literature it is mentioned that highly vigorous individuals and tolerance of herbivory are two relevant factors explaining the invasion success (Gard *et al*., 2011; Bonini *et al*., 2016). On the other hand, Ambrosia has been shown to respond favourably to warming and nitrogen addition (Essl *et al*., 2009). Some results confirmed that water supply plays important role in its invasivity. In combination with disturbance and other environmental stressors which decrease the competition intensity, Ambrosia might further spread into more productive environments (Leskovsek *et al*., 2012). The explosive spread could be facilitated by arbuscular mycorrhizal fungi (Fumanal *et al*., 2006). Another very important aspect is that common ragweed has developed resistance to linuron, S-triazine herbicides and many Group 2 herbicides (Saint-Louis *et al*., 2005). Invasiveness and high plasticity may be related to the germination behaviour of seeds. For example, the seeds from roadside soils is better adapted to the high salinity concentrations than seeds from agricultural field populations (DiTommaso, 2004).

The potential area of *Ambrosia artemisiifolia* distribution is at latitudes of 50°-55°N. These borders could enlarge because of acclimatization in new regions or reduce due to control and quarantine measures. We hope that the control measures recently adopted in Law 62/2018 against *Ambrosia* will reduce the invasive plants populations and consequently will decrease the atmospheric pollen load (Leru *et al*., 2014; Leru *et al*., 2019). Uncontrolled spread of this invasive plant all over the country is a serious threat to human health. This information is important both in relation to the forecasting of *Ambrosia* pollen concentrations in the ambient air but also in the design of mitigation strategies because effective strategies vary with the distribution and abundance of plants. A study like this has never been taken before for this long period and this invaded area.

Conclusions

This study has an important contribution for identification of levels of *Ambrosia artemisiifolia* pollen in Romania. The data from the entire 10-year study were analysed. Pearson's correlation test and multiple regression analyse were employed to determine the degree of dependence between the pollen concentrations and meteorological conditions. The *Ambrosia* pollen load of Timisoara is most important between 15 August - 15 September. Our data confirm that *Ambrosia* pollen is actually a major allergen for the urban environment and for the Western region of Romania also. A longer monitoring period and correlation with health data from allergists are needed. The aerobiological monitoring of this aeroallergen may be useful for the local and national authorities, the clinicians, the patients, and the general public in the Romania. Beyond the certain influences of the meteorological factors, our data illustrate that there has been a gradual increase in pollen concentrations from year to year. It can be stated that the rising trend of the seasonal pollen index reflects the expansion of *Ambrosia* populations.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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