



An assessment of ragweed pollen and allergen loads in an uninvaded area in the Western Black Sea region of Turkey

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Abstract *Ambrosia artemisiifolia* L., known as common ragweed, is an invasive and allergenic plant that has begun to expand its presence in Turkey. Although ragweed pollen has been monitored for a long time—especially in the atmosphere of cities in Northern Anatolia—the invasion of the plant is more limited compared to that of other European countries. Here, we present the highest ragweed pollen concentration recorded in Turkey, as well as changes in the *Amb a 1* allergen level in the air during 2015 and 2016 in Zonguldak, measured using a BGI 900 cascade impactor. The Seasonal Pollen Integral (SPI_n) was found to be 709 pollen day/m³ and 1542 pollen day/m³ in 2015–2016, respectively. The sum of *Amb a 1* in both stages was 8.42×10^{-3} U/m³ and 13.1×10^{-3} U/m³ over the same period. The number of days in which the *Ambrosia* pollen concentration was higher than 50 pollen/m³ doubled in 2016

compared to 2015, despite the negative effect of rainfall on pollen and allergen concentration in 2016. The pollen and allergen sources were evaluated by using simulated air mass movement with a Hybrid Single-Particle Lagrangian Integrated Trajectory model. Our findings suggest that individuals in Zonguldak are exposed as much to pollen—and possibly allergens—transported from the Ukraine, Russia and to a limited extent from Moldova. Furthermore, the allergen content of pollen in Zonguldak was much higher than other cities where *Amb a 1* is monitored. We also demonstrated that the date of the peak pollen day may be an indicator of the source of long distance transportation.

Keywords Ragweed · Pollen · Allergen · *Amb a 1* · Monitoring · Long distance transportation

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1 Introduction

Common ragweed (*Ambrosia artemisiifolia*) from the Asteraceae family is an annual and highly allergenic invasive plant (Hamaoui-Laguel et al. 2015). Historical records show that there are three species (*Ambrosia maritima*, *Ambrosia tenuifolia* and *A. artemisiifolia*) in Turkey (Önen 2015). As in Europe, *Ambrosia maritima*—belonging to the genus *Ambrosia*—is the only species that has a natural spread in Turkey. *A. maritima* spreads across the Mediterranean

region (Antalya, Konya, Adana and Hatay Provinces) (Kupicha 1975). The *Ambrosia tenuifolia* species was observed on the road and field edges in Malatya in 2000 (Behçet 2004). After common ragweed was first found in Europe in the nineteenth century, it expanded its presence. *Ambrosia* spreads initially along river banks, then to agricultural fields and finally along roadsides and railways (Yair et al. 2019). Besides the most infected areas in Europe, such as the Pannonian Plain, the Rhone–Alps region of France and parts of Northern Italy (Makra et al. 2015; Smith et al. 2013), there are also some reports of common ragweed being found in Turkey (Önen et al. 2014; Özaslan et al. 2016). The first report was published by Byfield and Baytop (1998) in Trabzon. After that, Özaslan et al. (2016) also observed ragweed plants in sunflower fields in the Thrace region of Turkey. Even though Celenk (2019) and Celenk and Malyer (2017) reported that Bursa city is located 400 km from the northwestern edge of the distribution area of *A. artemisiifolia*, Bıçakçı and Tosunoglu (2015) observed the common ragweed plant in Düzce city, which is approximately 200 km from İstanbul and Bursa. A more recent study reported that 41 different common ragweed populations had been found, and their sizes ranged from 1 to 4375 m² in Düzce (Zambak and Uludag 2019). In addition, there are records of *A. artemisiifolia* in the Eastern Black Sea region (Aksoy et al. 2010; Aslan et al. 2013; Serbes et al. 2008). The settlements of common ragweed plant in the Eastern Black Sea region of Turkey originated from Turkey's neighbors to the northeast, probably from Georgia (Özaslan et al. 2016). It has been also speculated that the expansion of ragweed into Turkey is linked to the construction of the Black Sea Highway, because that construction cleared all native plants from the land and created suitable conditions for the invasion of the weed (Önen et al. 2014). Although the plant has been recorded in Northern Turkey, Central Anatolia is still considered to be ragweed-free (Önen 2015).

The higher pollen and seed production capabilities of ragweed allow it to expand quickly (Fumanal et al. 2007). Alongside this, it has been noted in several studies that the prevalence of ragweed allergy has recently increased, and it is estimated that this could be worse in the near future as a result of global climate change (Lake et al. 2016). There is speculation about the sources of pollen which are observed in Turkey. Using the backward trajectory model on a peak pollen

day, Zemmer et al. (2012) reported that *Ambrosia* pollen, which is observed in the atmosphere of İstanbul, could have migrated from Bulgaria, the Ukraine, Moldova or the Russian coastal region of the Black Sea. Long distance transportation of ragweed pollen was also noted by Celenk and Malyer (2017) via pollen monitoring studies in Bursa. Celenk (2019) also monitored *Amb a 1* allergen and pollen concentrations in Bursa and emphasized that *Ambrosia* pollen allergens could be active even after being transported for at least 48 h. The high allergenic capacity of ragweed pollen grains (D'Amato et al. 2007) means that low concentrations of *Ambrosia* pollen can be important for allergy sufferers, although the clinical relevance of the long distance transportation (LDT) of allergenic ragweed pollen remains unclear. Ongoing findings suggest a limited effect on the short-term risk of new sensitizations to ragweed pollen, but the increasing number of reports on the occurrence of long distance pollen transportation suggests that more studies are required in the future (Cecchi et al. 2010; Sommer et al. 2015).

Based on field observations (personal observations from visits to about 200 locations during 2017–2018) of ragweed in Zonguldak, the province could be considered ragweed-free for now. On the other hand, the plant has been reported in Düzce which is 85 km from Zonguldak (Bıçakçı and Tosunoğlu 2015). In our study, focusing on the determination of the contribution of long distance transportation of pollen to the allergen loads, we have combined the analysis of monitoring both *Ambrosia* pollen and *Amb a 1* allergen concentrations with a back trajectory analysis of air masses during 2015–2016 in Zonguldak.

2 Materials and method

2.1 Study area

The study was carried out in Zonguldak, a city with a population of about 110,000 (Fig. 1). Zonguldak is located in the Euro-Siberian phytogeographic region. Due to its hilly topography, the settlement is irregular and ends at the coastline. The natural vegetation of Zonguldak consists mainly of deciduous forests. Showing great deviation from the seasonal norms, the average annual rainfall is 1040.5 mm and the annual average temperature is 13.6 °C, according to

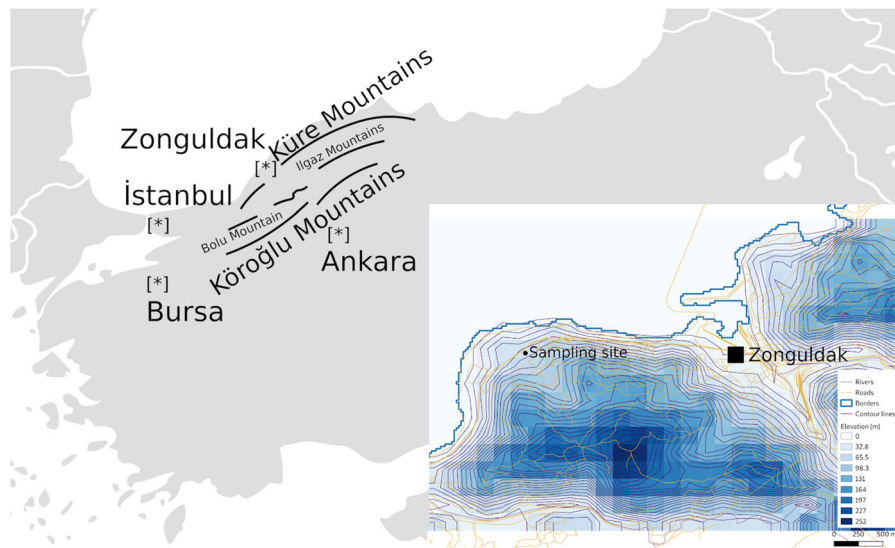


Fig. 1 Location of Zonguldak and important mountains in the Western Black Sea Region of Turkey along with the major cities of İstanbul, Ankara and Bursa, and showing a contour line map of Zonguldak city center (in frame)

data obtained from the Turkish State Meteorological Service station in Zonguldak. Daily meteorological data: daily mean temperature ($^{\circ}\text{C}$), relative humidity (%), total rainfall (mm) and wind speed (m/s), were obtained from the central meteorological station situated at Zonguldak (approximately 1.5 km south-east of the monitoring station), which belongs to the network of stations run by the Turkish State Meteorological Service.

2.2 Pollen sampling and counts

A 7-day, volumetric Hirst-type pollen and spore trap was used for the collection of atmospheric *Ambrosia* pollen during 2015–2016. Sampling was performed using the standard protocol prepared by the Spanish Aerobiology Network (REA) (Galán et al. 2007) and the minimum recommendations of the European Aeroallergen Network (Galán et al. 2017). Then, the amount of pollen was expressed in pollen/ m^3 by using the technique of counting the full area of the slide. The pollen season was split into episodes according to remarkable increases in *Ambrosia* pollen concentration (5 pollen/ m^3).

2.3 Quantifying and sampling of allergens

Amb a 1 in the air was sampled using polyurethane filters and a BGI 900 (Mesa Labs, USA) high volume

cascade impactor was equipped with two different particulate matter (PM) stages: greater than $10\ \mu\text{m}$ ($\text{PM} > 10$) and $10\ \mu\text{m} > \text{PM} > 2.5\ \mu\text{m}$ ($10 > \text{PM} > 2.5$). Both samplers were placed on the roof of a building in Zonguldak Bülent Ecevit University Campus at a height of approximately 30 m with a distance of 5 m between them. They were operated simultaneously in the pollen season (July to October).

The extraction procedure is described by Alan et al. (2018); Buters et al. (2012). Briefly, the filters were cut into three identical parts. One of them was stored as a backup at $-20\ ^{\circ}\text{C}$. The remainder was extracted in a carbonate buffer for 4 h in the dark. After separation by centrifugation and condensation by lyophilization, the concentration of *Amb a 1* was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using polyclonal antibodies (Indoor Biotechnologies). The *Amb a 1* allergen concentration was expressed as U m^{-3} . The enzyme unit, U, is the amount of enzyme that converts one micromole of substrate per minute according to the kit manufacturer's manual. In order to compare our results with other studies in which pg (picogram) was used as the units, it was assumed that one unit equals one microgram (Cox et al. 2007; Lockey and Ledford 2014).

2.4 Statistical analysis

Only the dates on which allergens and pollen were observed were selected for correlation analysis. As the full dataset did not fit a normal distribution (results not given), the nonparametric Spearman rank correlation test was performed to investigate the relationship between the pollen and the allergen level, as well as with the meteorological parameters. The Mann–Whitney *U* test was used for a comparison of the pollen and allergen dataset obtained from the different years (Aboulaich et al. 2013). While the SciPy python package was used for all statistical analyses (Jones et al. 2014), the R statistical programming language (Team 2013) was used for the preparation of all graphics. Polar plots showing pollen and allergen distribution according to actual wind speed and direction at the sampling site were carried out using the openair package (Ropkins and Carslaw 2012). Since allergen and pollen concentration did not overlap, no threshold value was chosen for either pollen or allergen.

2.5 Back trajectory analysis

The Hybrid Single-Particle Lagrangian Integrated Trajectory model (HYSPPLIT) developed by the National Oceanic and Atmospheric Administration (NOAA) was used to determine the possible sources of air masses reaching Zonguldak. Days on which the highest pollen and allergen concentrations were observed for each episode, and the season, were chosen for the analysis in both years (Table 3). Data from the Global Data Analysis System (GDAS) archives were used as input for the calculation (with a spatial resolution of 1°). Trajectories were calculated 48 h back in time for every 6 h. (Stein et al. 2015). The back trajectories were calculated on an episodic basis in order to determine whether the source of pollen or allergen caused the episodes. Since *Amb a 1* was analyzed daily, daily resolution of pollen data was used. Because pollen and allergen concentration data have a different resolution (amount of *Amb a 1* was measured daily resolution), the time that the filters were changed was selected as arrival time. The air masses on peak days for episodes of both pollen and allergens are shown on the same plot to distinguish whether their possible sources were the same or not.

As the samplers were placed very close to the seaside, there were no obstacles to prevent air masses reaching the samplers. Therefore, the starting elevation was 50 m above ground level (AGL). Trajectories were calculated at Zonguldak with a height of 50 m AGL.

3 Results

3.1 Pollen data

The Seasonal Pollen Integrals (SPIn) for 2015 and 2016 were 709 pollen day/m³ and 1542 pollen day/m³, respectively. The major pollen peak in 2015 was on August 31 (184.9 pollen/m³). Risk days for sensitive patients are defined according to Portnoy and Barnes (2003) as days with a pollen concentration of more than 50 pollen/m³. SPIn and the number of risk days were twice as high in 2016 than in 2015, while the concentration on the peak day did not increase as much (Table 1). The highest pollen concentration was obtained on August 25 (249.6 pollen/m³) (Table 1).

3.2 Airborne pollen allergens

The sum of *Amb a 1* in both in stages in 2015 and 2016 was 8.42×10^{-3} U/m³ and 13.1×10^{-3} U/m³, respectively. The peak allergen observation dates were August 30, 2015, and August 31, 2016 (Table 1). A positive correlation was found between pollen and the sum of *Amb a 1* during 2015 and 2016 (Table 2). In addition, the peak value in 2015 was higher than in 2016. Another notable finding about the peak pollen and allergen days was the timing of the peak allergen date. While in 2015, the allergen peak was recorded one day earlier than the pollen peak, in 2016, the allergen peak was recorded six days later than the pollen peak.

3.3 Meteorological factors

It was found that there was a positive correlation between pollen and allergen levels during 2015–2016. When the correlation levels among the pollen and allergens collected by the two stages of the impactor were compared, the correlation degree between allergens and pollen from 10 > PM > 2.5 was slightly higher than those from PM > 10 in both years

Table 1 Pollen and allergen contents of the atmosphere in Zonguldak during 2015 and 2016

Year	Season	Pollen				Allergen		
		SPIn (pollen day m^{-3})	Peak day	Peak value (P m^{-3})	Number of days with > 50 P m^{-3}	Sum of <i>Amb a l</i> in both in stages ($\text{U m}^{-3}/$ season)	Peak day (U m^{-3})	Peak value (U m^{-3})
2015	03.08/20.09	709	31.08	184.9	4	8.42×10^{-3}	30.08	3.06×10^{-3}
2016	28.07/20.09	1542	25.08	249.6	8	13.1×10^{-3}	31.08	1.71×10^{-3}

Table 2 Results of a correlation analysis between pollen, allergens and meteorological parameters in Zonguldak

Parameters	2015				2016			
	Pollen	Allergen			Pollen	Allergen		
		PM > 10	10 > PM > 2.5	Sum of <i>Amb a l</i> in both stages		PM > 10	10 > PM > 2.5	Sum of <i>Amb a l</i> in both stages
SPIn	1.0	0.42**	0.589**	0.622**	1.0	0.423**	0.454**	0.437**
PM > 10	0.42**	1.0	0.513**	0.938**	0.423**	1.0	0.722**	0.998**
10 > PM > 2.5	0.589**	0.513**	1.0	0.737**	0.454**	0.722**	1.0	0.741**
Sum of <i>Amb a l</i> in both stages	0.622**	0.938**	0.737**	1.0	0.437**	0.998**	0.741**	1.0
Mean. T.	− 0.06	0.151	− 0.122	0.218	− 0.199	− 0.009	0.083	− 0.012
Min. T.	− 0.149	0.103	− 0.294	0.059	− 0.069	0.025	0.022	0.019
Max. T.	− 0.019	0.244	0.044	0.313*	− 0.275*	0.01	0.025	0.006
Air Pr.	0.63**	0.06	0.424*	0.24	0.31*	0.15	− 0.042	0.155
Rel. Hu.	− 0.311*	− 0.054	− 0.45**	− 0.348*	0.069	0.14	0.066	0.14
Wind S.	0.545**	0.127	0.307*	0.23	0.051	0.065	0.131	0.067
Wind dir.	0.148	0.02	0.102	0.06	− 0.094	0.183	0.141	0.183
Rainfall	− 0.061	− 0.011	− 0.071	− 0.036	0.108	− 0.089	− 0.181	− 0.094

* $p < 0.05$, ** $p < 0.01$

(Table 2). Three different episodes could be distinguished in the pollen and allergen seasons in both years (Fig. 2). The distinction between these episodes was clearer in 2016 than in 2015 (Table 3). The main season in Zonguldak in 2016 was interrupted by strong rainfall observed from August 26 to August 28. In addition, the majority of pollen and allergen concentrations in both years were observed in Episode II (Table 3).

Only air pressure was found to be correlated with pollen in both years. On the other hand, the changes in pollen and allergen concentrations in 2015 were more correlated with meteorological factors than those of

2016. Although rainfall had no statistically significant correlation to pollen and allergens in 2015, it was effective on pollen and allergen concentrations in 2016. Considering the distribution of the pollen season during 2015 and 2016, it was found that the pollen season in 2015 had a more compact distribution, while a significant portion of pollen was observed after the main pollen season in 2016. This was not observed for allergens. The wind speed showed a positive correlation to pollen grains in 2015, but it was not detected in 2016. In addition, wind direction was also examined, and polar plots were created for both pollen and allergen levels in 2015–2016. There was no significant

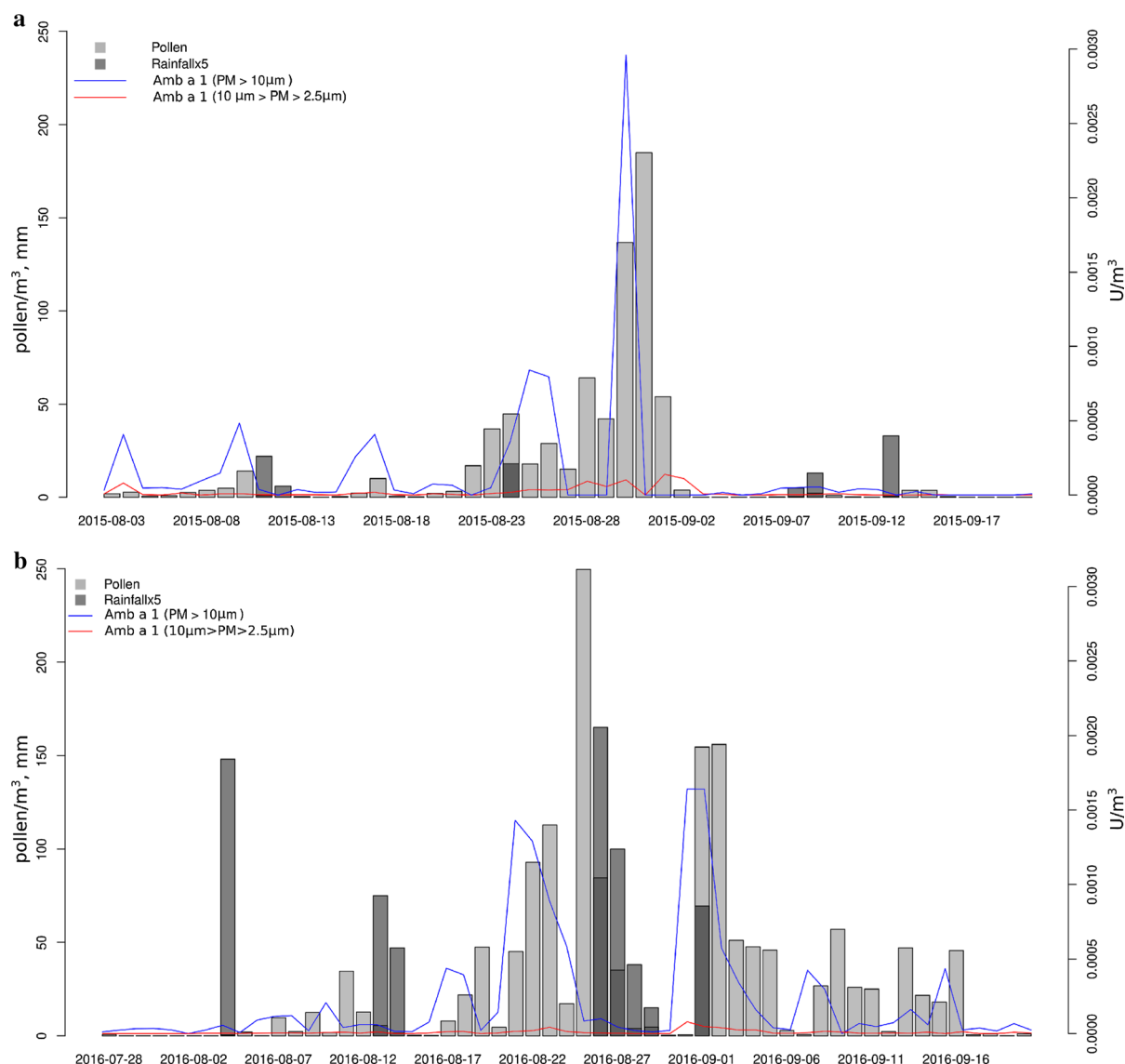


Fig. 2 Variations in daily concentrations of ragweed pollen, *Amb a 1* and rainfall (darker bars) in Zonguldak in 2015 (a) and 2016 (b)

correlation between the wind directions and pollen and allergen concentrations. After all, it was determined that the winds blew from a southeast direction on the peak days of both pollen and allergens in 2015 (Fig. 3). Similarly, on all 25 days when airborne ragweed pollen concentrations were greater than 10 pollen/m^3 , it was determined that the winds were blown from the southeast. However, the majority of pollen and allergen concentrations were monitored on days in which the wind blew from many different directions in 2016 (Fig. 3a, b).

The results of the Mann–Whitney U test indicated that there was a statistically significant difference in the mean daily pollen and allergen concentration from the $\text{PM} > 10$ filters in 2015 compared to 2016 (Table 4). On the other hand, allergen concentrations from $10 > \text{PM} > 2.5$ filters showed similar distribution in both years.

3.4 Back trajectory analysis

Back trajectory analysis revealed that the *Ambrosia* pollen and *Amb a 1* concentration, which were

Table 3 Summarizing characteristics of episodes observed in Zonguldak during 2015 and 2016

	2015			2016		
	Episode I	Episode II	Episode III	Episode I	Episode II	Episode III
Dates	03.08–11.08	12.08/03.09	04.09/20.09	28.07/16.08	17.08/07.09	08.09/20.09
Pollen Integral (pollen day/ m^{-3})	31.8 (4.5%)	664.4 (93.7%)	12.6 (1.8%)	83.7 (5.4%)	1186.3 (77%)	271.6 (17.6%)
PM > 10 (U/ m^{-3})	1.34×10^{-3} (17.7%)	5.93×10^{-3} (78.2%)	3.08×10^{-4} (4.1%)	1.04×10^{-3} (8.2%)	10.3×10^{-3} (81.1%)	1.31×10^{-3} (10.7%)
10 > PM > 2.5 (U/ m^{-3})	1.25×10^{-4} (14.7%)	6.94×10^{-4} (81.7%)	3.14×10^{-5} (3.6%)	5.89×10^{-5} (11.9%)	3.63×10^{-4} (73%)	7.45×10^{-5} (15.1%)
Sum of allergen in both stages (U/ m^{-3})	1.47×10^{-3} (17.5%)	6.62×10^{-3} (78.6%)	3.39×10^{-4} (3.9%)	1.10×10^{-3} (8.4%)	10.7×10^{-3} (81.7%)	1.39×10^{-3} (9.9%)
Mean Temp. (°C)	23.1	22.6	21.2	24	22.8	21.9
Humidity (%)	85	77.5	80.8	87.4	85.7	76
Pressure (mmHg)	997.3	913.2	–	997.4	998.8	998
Rainfall (mm)	4.4	4.8	10.2	54	77.5	0
Pollen peak day	10.08	31.08	14.09	11.08	25.08	09.09
Allergen peak day	10.08	30.08	09.09	10.08	31.08	15.09

recorded on the peak days, could have been transported from the Ukraine, Russia and Moldova (Fig. 4). It was found that air masses on the peak pollen and allergen days in 2015 were transported from the Crimea region (Fig. 4a, b); in 2016, peak pollen days in every episode originated from different parts of the Ukraine which belong to regions with different *Ambrosia* pollen loads (Rodinkova et al. 2018). Back trajectory analysis clearly indicates that the air mass on peak allergen days did not share the same routes as those of the peak pollen days in 2016. It seemed that some of the trajectories in Episode III for both pollen and allergens in 2015 came from the Düzce region (regional source) where the ragweed population had already localized (Fig. 4a, b).

4 Discussion

The SPIn presented in this study for Zonguldak in 2016 was the highest value recorded among the studies in Turkey (Supplementary Tab. S1). This was followed by Samsun (1284 pollen day/ m^3) (Bıçakçı and

Tosunoğlu 2015). Celenk (2019) and Celenk and Malyer (2017) reported that the highest *Ambrosia* pollen concentration of 878 pollen day/ m^3 in 2010–2015 was in Bursa. Moreover, when compared with other studies, especially for regions in Europe that have been invaded, our values are still very high. Skjøth et al. (2013) prepared a pollen distribution map of Europe for *Ambrosia* with ten different categories. If the two-year SPIn of Zonguldak was classified according to this scale, it would be comparable with Poland. A similar pollen distribution map was prepared for the Ukraine for 2010 (Rodinkova et al. 2018). According to this map, the majority of the population in southeast Ukraine is subjected to a total pollen load of 3000–10,000 grains/ m^3 per season. In the forest steppe, the total *Ambrosia* pollen load was 1000–3000 grains/ m^3 per season and this load is similar to our results for 2016.

There is no reference for the presence of ragweed in Zonguldak, and also, the plant has not been observed in personal field trips in Zonguldak. Considering these, it is a striking result to be monitoring such a high pollen concentration. Our results indicated that all

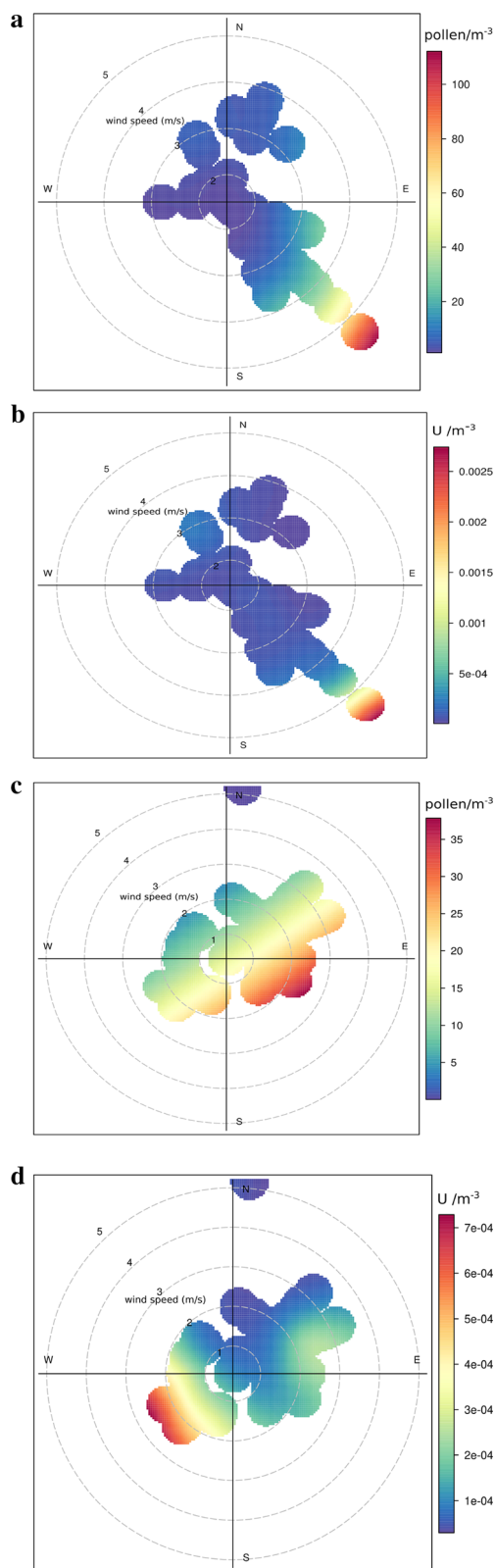


Fig. 3 Polar plot of pollen ($P \text{ m}^{-3}$) and allergen ($U \text{ m}^{-3}$) concentrations to show the distribution of pollen and allergens based on wind speed and direction. **a, b** pollen and allergen in 2015, **c, d** pollen and allergen in 2016

pollens observed in Zonguldak were transported by regional or long distance transportation.

There are many different threshold values to express pollen concentration that trigger an allergic reaction. Kasprzyk et al. (2011) reported that an *Ambrosia* pollen concentration higher than 20 pollen/ m^3 was recorded on seven days in 2002 in Rzeszów (Poland) where the nearest sites of *A. artemisiifolia* were observed to be about 70 km away, similar to Zonguldak. Although the threshold value was accepted to be 50 pollen/ m^3 in our study, the number of days on which *Ambrosia* pollen concentration was higher than this threshold value was 4 and 8 days, respectively, during 2015–2016.

Back trajectory analysis also revealed that the pollen and *Amb a 1* concentration originated from similar possible sources (mainly the Ukraine and Russia) in 2015. In 2016, even though the source of air masses on peak allergen days was the Ukraine region with the highest ragweed infestation, air masses on peak pollen days were mainly transported from western Ukraine and Moldova. While greater pollen and allergen concentrations were recorded in 2016 compared to 2015, the peak allergen value in 2016 was lower than in 2015. On the other hand, the peak pollen value in 2016 was higher than in 2015, consistent with a higher SPIn in 2016. Unusual date differences between pollen and allergen peak days support this hypothesis. The distribution of pollen and allergens according to wind direction showed a different pattern in 2016 (Fig. 3c, d), which could be another indicator that pollen and allergen peak days could be independent of each other.

Although the results of polar plots indicated that both pollen and allergen concentrations were recorded in the opposite direction to the possible source of long distance transportation—with air masses mainly coming from the north—they may not have reached the samplers directly due to the hilly topography of Zonguldak in that direction. Instead, the air masses could have reached the samplers after passing around the hills surrounding the sampling site (Fig. 1).

Table 4 Mann–Whitney *U* test results for pollen and allergen concentrations

	Pollen	PM > 10	10 > PM > 2.5	Sum of <i>Amb a 1</i> in both stages
<i>p</i> value	0.02924*	0.02561*	0.8411	0.04514*

**p* < 0.05

The date of the peak day may be another indicator in estimating the sources of long distance transportation. There are many different reports for the date of the *Ambrosia* peak pollen day in Turkey. While Zemmer et al. (2012) observed the peak day on August 28, Celenk and Malyer (2017) reported that the peak day occurred between late August and September and varied across the years in Bursa. Another study showed that *Ambrosia* pollen observed in Bursa could have been transported by air masses moving over Zonguldak (Bıçakçı and Tosunoğlu 2015). Celenk and Malyer (2017) also determined that the source of *Ambrosia* pollen was Russia for the years in which the peak day was observed to be in late August (Celenk and Malyer 2017). Our results supported these findings. In Zonguldak, the date of the peak days for *Ambrosia* pollen has been consistent over the years (Kaplan et al. 2003). Synchronization of pollen seasons in different places may have been caused by transport of pollen from the same distant sources. Thus, it can be said that observing the pollen peak at the end of August may be an indication that the pollen was transported from Russia and the Ukraine. Similarly, Rodinkova et al. (2018) showed that the peak days in the Ukraine correspond to the end of the August, based on the pollen season data from different monitoring sites in 2009–2016 (Rodinkova et al. 2018). Although the peak pollen days were observed earlier in 2016 than in 2015, this may have been associated with rainfall as described earlier. In addition, the allergen peak value measured in 2015 was higher than in 2016, whereas the seasonal sum of allergen content in 2016 was higher than in 2015. It is also a clear indication that rainfall has a negative effect on allergen concentrations as well as on pollen in the air.

Although they were not defined as episodes by the authors, three occurrences similar to our results have been seen in Istanbul (Zemmer et al. 2012). Episodes for *Ambrosia* were also defined by other authors for Poznań (Grewling et al. 2016) and Bursa (Celenk

2019). The pollen and allergen peaks were observed at the end of August in both Poznań and Zonguldak. These authors also defined three episodes in total, in which the first episode was the main pollen and allergen season corresponding to our second episode. This situation could be associated with phenological differences between aerobiological stations due to their location at different latitudes.

Grewling et al. (2016) also reported concentrations of *Amb a 1* and *Ambrosia* pollen in the atmosphere of Poznań, which like Zonguldak is considered to be an uninfected area. They recorded 937.8 pg of *Amb a 1* in 2011. The unit that we used in this study to represent the allergen level was different from that previous study. After conversion of our results, the sum of *Amb a 1* was 8430 pg/m³ and 13,200 pg/m³ in Zonguldak during 2015 and 2016, respectively. The allergen loads of Zonguldak were significantly higher than those of Poznań. Studies have shown great differences between countries with cumulative Bet v 1 concentration and pollen potency for Phl p 5 (Buters et al. 2012; 2015). In this study, similar differences were observed despite the different years studied. Grewling et al. (2016) also emphasized that Poznań is 250 km away from large ragweed populations. Another reason, based on the back trajectory model, is that the source area of *Ambrosia* pollen of Zonguldak is most probably Russia and the Ukraine where the highest *Ambrosia* pollen concentrations have been recorded. Besides that, the distribution of pollen concentrations in the season was different from Grewling et al. (2016). While three short episodes were observed in Poznań, that pollen concentration was observed almost everyday in Zonguldak. Considering that some trajectories, especially in Episode III in 2015, it could be evaluated that regional pollen transportation from Düzcce has a limited contribution to the pollen and allergen loads of Zonguldak.

Although similar pollen concentrations were recorded between Zonguldak and Bursa in 2015, pollen and allergen values in Zonguldak during

Fig. 4 48-h backward trajectories calculated every 6 h from Zonguldak at 50 m at 12:00 (UTC + 3) for each calculated day: **a** the peak pollen days of each episode in 2015, **b** the peak allergen days of each episode in 2015, **c** the peak seasonal pollen and allergen days in 2015, **d** the peak pollen days of each episode in 2016, **e** the peak allergen days of each episode in 2016 and **f** the peak seasonal pollen and allergen days in 2016

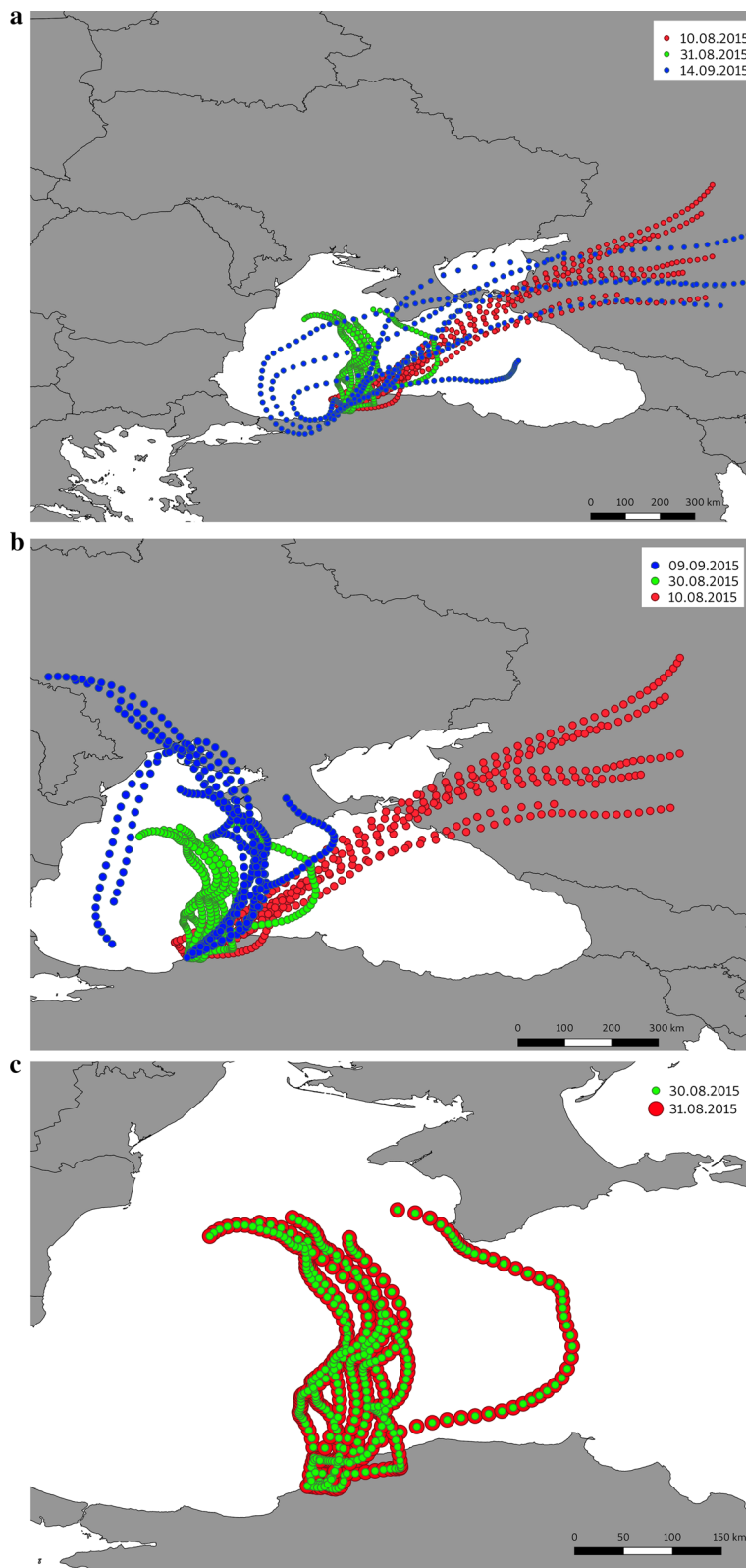
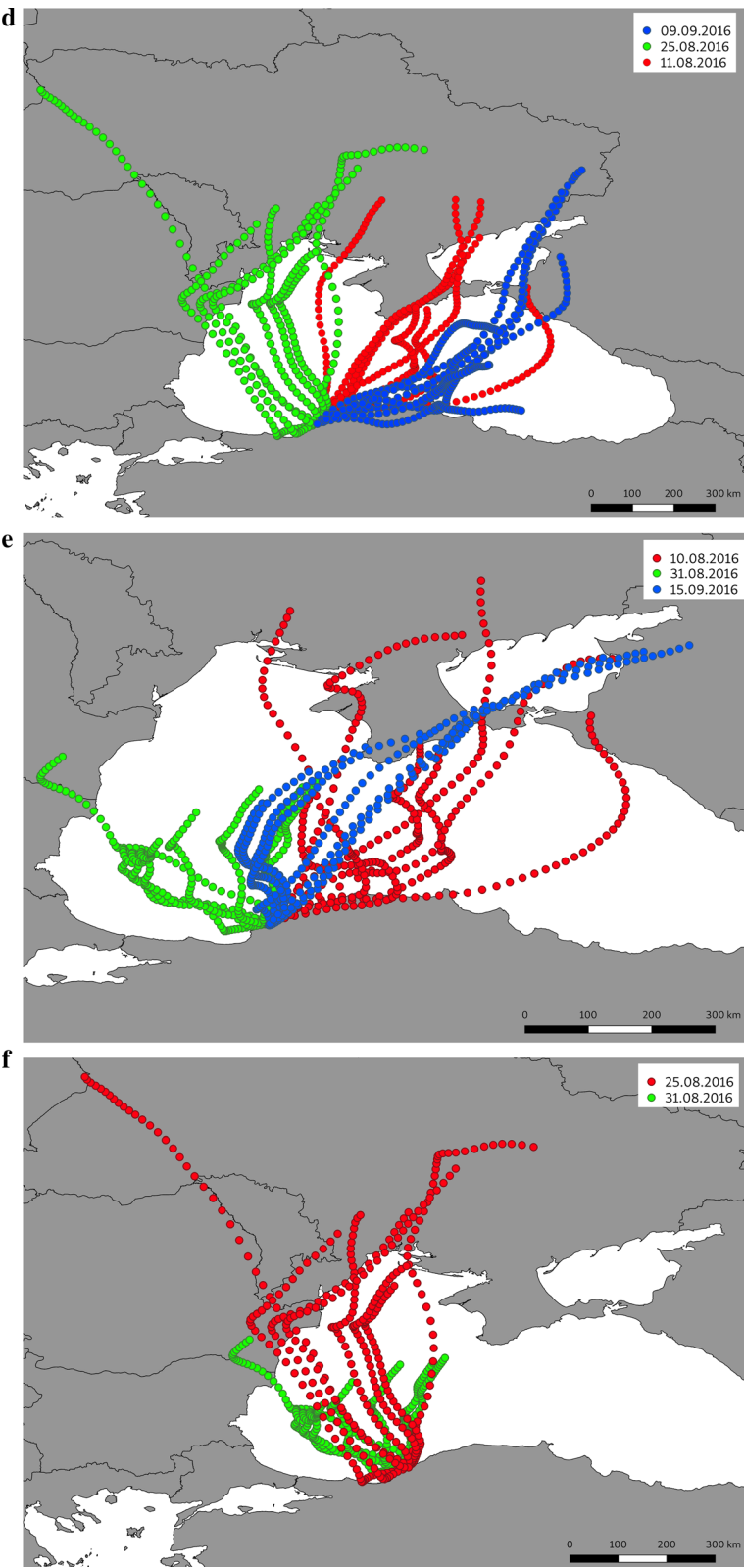


Fig. 4 continued



2015–2016 were significantly higher than those of Bursa in 2014. Given that there are insufficient studies on *Amb a 1*, it is very hard to compare the data over the years. It appears that the $10 > \text{PM} > 2.5$ allergen sources were similar in 2015 and 2016 due to Mann–Whitney *U* test. A considerable amount of $10 > \text{PM} > 2.5$ allergens was observed before the main season. Zonguldak is located at a lower latitude than the possible long distance sources of ragweed, and thus, the release of *Amb a 1* (pectate lyase) allergens during anthesis or pollen tube growth can be expected to occur earlier than in the higher latitudes. This study showed that meteorological factors such as rainfall can directly affect pollen and allergen concentrations even if there is no statistically significant correlation between them. Furthermore, long distance transportation (LDT) could cause sensitized patients to be subjected to an exposure comparable to the high amount of pollen allergen concentrations within invaded regions.

5 Conclusions

In conclusion, while the *Ambrosia* pollen observed in Zonguldak in Episode II must have been long distance transported from the Ukraine, Russia and Moldova, pollen and allergens in Episodes III might have been transported from regional sources. The doubling of the number of risky days for sensitive patients over the years could be evaluated by people developing more symptoms than normal. Allergen and pollen monitoring studies over the same period, and in areas where the same air masses flow, will help to determine the changes in the allergenic activity of the transported pollen.

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Supplementary Table. S1. SPIn values of *Ambrosia* pollen in some locations from Turkey.

<i>Locations</i>	<i>Pollen integral</i>	<i>Year /average</i>
<i>Samsun*</i>	1284	2005
<i>Yalova*</i>	738	2005
<i>Edirne*</i>	737	2006
<i>Mudanya*</i>	645	2006
<i>Bursa**</i>	565	2010-2015
<i>Kocaeli*</i>	459	2012
<i>İstanbul*</i>	454	2013
<i>Ankara*</i>	250	1990-1999

*Bıçakçı and Tosunoğlu 2015, ** Çelenk 2019