Is the recent decrease in airborne *Ambrosia* pollen in the Milan area due to the accidental introduction of the ragweed leaf beetle *Ophraella communa*?

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Abstract This study aims to determine whether a significant decrease in airborne concentrations of *Ambrosia* pollen witnessed in the north-west of the Province of Milan in Northern Italy could be explained by environmental factors such as meteorology, or whether there is evidence to support the hypothesis that the decrease was related to the presence of large numbers of the oligophagous *Ophraella communa* leaf beetles that are used as a biological control agent against *Ambrosia* in other parts of the world. Airborne concentrations of *Ambrosia*, Cannabaceae and Urticaceae pollen data (2000–2013) were examined for trends over time and correlated with meteorological data. The amount of *Ambrosia* pollen recorded annually during the main flowering period of *Ambrosia* (August–September) was entered into linear regression models with meteorological data in order to determine whether the amount of airborne *Ambrosia* pollen recorded in 2013 was lower than would normally be expected based on the prevailing weather conditions. There were a number of significant correlations between concentrations of airborne *Ambrosia*, Cannabaceae and Urticaceae pollen, as well as between airborne pollen concentrations and daily and monthly meteorological data. The linear regression models greatly overestimated the amount of airborne *Ambrosia* pollen in 2013. The results of the regression analysis support the hypothesis that the observed decrease in airborne *Ambrosia* pollen may indeed be related to the presence of large numbers of *O. communa* in the Milan area, as the drastic decrease in airborne *Ambrosia* pollen in 2013 cannot be explained by meteorology alone.

Keywords Aerobiology · Ragweed · *Ophraella communa* · Biocontrol agent

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1 Introduction

Ambrosia maritima L. is the only *Ambrosia* (ragweed) species native to Europe; three other species of *Ambrosia* (*A. psilostachya*, *A. tenuifolia* and *A. trifida*) were introduced to Europe from North America in shipments of agricultural products (Smith et al. 2013). Of these introduced species, the invasive alien *Ambrosia artemisiifolia* L. (common or short ragweed) is the most widely distributed and as such is considered to be an import weed in agriculture and source of highly allergenic pollen, which causes symptoms of pollen allergy in late summer and autumn and reportedly induces asthma about twice as often as other pollen types (Dahl et al. (1999) and references therein).

The Rhône Valley (France), parts of Northern Italy, the Pannonian Plain (i.e. predominantly Hungary but also parts of Austria, Bosnia and Herzegovina, Croatia, Czech Republic, Romania, Serbia, Slovakia and Slovenia) and Ukraine are considered to be the most important sources of *Ambrosia* pollen in Europe (Smith et al. (2013) references therein). *Ambrosia artemisiifolia* is an anemophilous (wind pollinated) plant, the pollen grains of which can readily become airborne when conditions are favourable. As with other wind-pollinated plants, such as those belonging to the Cannabaceae and Urticaceae families that flower at around the same time as *Ambrosia*, pollen release and dispersal is aided by high temperature and low relative humidity (Dahl et al. (2013) and references therein).

Ophraella communa LeSage 1986 (Coleoptera: Chrysomelidae) is an oligophagous leaf beetle that feeds on various plants species belonging to the tribe Heliantheae (Asteraceae), including *A. artemisiifolia* (Futuyma and MCCafferty 1990; Palmer and Goeden 1991). The beetle originates from North America (Futuyma and MCCafferty 1990) and has been (accidentally) introduced to a number of other areas worldwide, including Europe, where it was first detected in 2013 (Takizawa et al. 1999; Meng and Li 2005; Müller-Schärer et al. 2014). Despite extensive host-specificity tests carried out over the past two decades (reviewed by Zhou et al. (2011)), there remains a controversial debate mainly on whether the beetle can attack and damage sunflower, *Helianthus annuus* L., in the field (cf. Müller-Schärer et al. (2014) and references therein). Therefore, host-specificity tests both under quarantine and open-field conditions in Switzerland and Northern Italy and including various sunflower varieties and *Ambrosia* species are presently underway (H. Müller-Schärer, unpublished results) to decide on whether this beetle should be considered a troublesome introduction or whether it is likely to become the first case of a successful biological control of an invasive weed in continental Europe.

Ophraella communa is multivoltine in that it produces three or four generations per year, with an egg-to-egg generation time of about 4 weeks (Futuyma et al. 1993). Females lay eggs every few days for a month or more, with the fecundity exceeding 2700 eggs/female (Futuyma et al. 1993; Zhou et al. 2010b). The survival rate of eggs and larvae is higher, and the development periods shorter, when temperature and humidity are increased (Zhou et al. 2010a, b; Zhu et al. 2012). Both the larva and imago feed on leaves of *A. artemisiifolia* (Wan et al. 2009). *O. communa* can significantly suppress plant height and the number of branches (Guo et al. 2011), and when beetle densities are high enough, it can even kill all *Ambrosia* plants (Zhou et al. 2014), thus preventing the production of seeds. Required densities are higher when plants are bigger, but even plants that were only experimentally exposed to the beetles when they were already over 90 cm tall were successfully killed prior to seed production (Zhou et al. 2014). In addition, *O. communa* has high dispersal potential (Tanaka and Yamanaka 2009). The flight distance has been estimated to be approximately 25 km/day, with potential range expansion of 329 km/year (Yamamura et al. 2007; Tanaka and Yamanaka 2009). Taking into account the selective host-feeding preferences, high fecundity and dispersal potential, it is suggested that *O. communa* may be a potential biological control agent against *A. artemisiifolia* (Teshler et al. 2002) although *Ophraella slobodkini* was a preferred candidate species for the biological control of *A. artemisiifolia* in Europe, because it exclusively feeds on *Ambrosia* (Gerber et al. 2011).

Ophraella communa beetles were observed feeding on *Ambrosia artemisiifolia* plants in several regions of Northern Italy, especially near Milan (Bosio et al. 2014; Müller-Schärer et al. 2014), during the summer of 2013. The beetle was found on all observed ragweed populations. Individual *A. artemisiifolia* plants harboured up to over a hundred beetles, and many plants were completely defoliated before the end of the season (Müller-Schärer et al. 2014). At the same time, it was noticed that routine pollen-monitoring stations situated in three towns of the north-west area of the Province of Milan were recording less *Ambrosia* pollen than usual. This study aims to determine whether the observed decrease in airborne concentrations of *Ambrosia* pollen can be explained by environmental factors such as meteorology that are known to strongly affect pollen release and dispersal, or whether there is evidence to support the hypothesis that the decrease was related to the presence of *O. communa* in the area.

2 Materials and methods

2.1 Airborne pollen data

Daily average pollen concentrations were collected at three sites in the north-west area of the Province of Milan, Northern Italy, from 2000 to 2013 (Fig. 1). Pollen data were collected by volumetric spore trap of the Hirst design (Hirst 1952). The pollen-monitoring sites were the towns of Legnano (ITMIL6), Magenta (ITMAGE) and Rho (ITRHO1). Legnano is the closest pollen-monitoring site to the meteorological stations, and so pollen data from this site were used throughout the analysis. Legnano is also the only pollen-monitoring site in this study where all three pollen types examined, *Ambrosia*, Cannabaceae and Urticaceae, are routinely monitored.

The amount of *Ambrosia* pollen recorded annually during the main flowering period of *Ambrosia* (August–September) is presented (Annual *Ambrosia* Pollen—AAP). The mean AAP of all three sites combined was also examined and termed “Milan area”. In addition, the amounts of Cannabaceae pollen (Annual Cannabaceae Pollen—ACP) and Urticaceae pollen (Annual Urticaceae Pollen—AUP) recorded during August–September at Legnano were included in the study as these taxa contain species that flower in August–September. As a result, the amount of these pollen types in the air can be assumed to be influenced by similar meteorological factors as *Ambrosia*. We therefore hypothesised that if airborne Cannabaceae and Urticaceae pollen concentrations do not follow a similar trend as *Ambrosia* pollen, i.e. the ACP and AUP did not decrease in the same way as AAP, then weather conditions during the period of pollen release are unlikely to be the main factor affecting airborne *Ambrosia* pollen concentrations. This would provide support for the hypothesis that the observed decrease in AAP was due to the presence of *O. communa* in the area, rather than meteorology. On the other hand, concurrent decreases in all three pollen types would

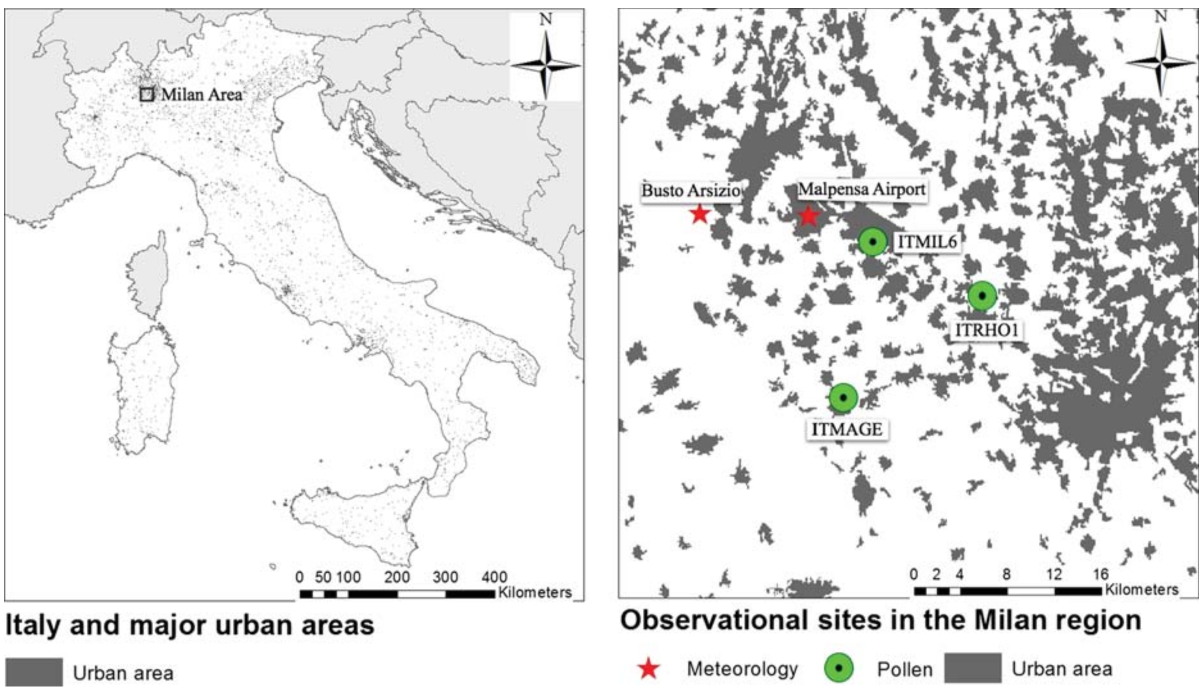

Fig. 1 Position of the pollen-monitoring sites and meteorology stations in the Milan region (Italy)

suggest that either conditions during August and September 2013 were generally not conducive for the release and dispersal of anemophilous pollen or that other factors which can affect the amount of pollen in the air from these three pollen types should be considered.

With this in mind, the following should be noted: (1) Cannabaceae and Urticaceae species tend to grow in similar habitats as *Ambrosia* (e.g. *Cannabis* on ruderal habitats and agricultural land, and *Urtica*/*Parietaria* on ruderal habitats); (2) All host-range tests conducted so far indicate that *O. communa* is specific to the Asteraceae tribe Heliantheae (Palmer and Goeden 1991; Gerber et al. 2011). As a result, Cannabaceae and Urticaceae populations could be affected by *Ambrosia* eradication measures in the Milan area, and concurrent decreases in airborne Cannabaceae and Urticaceae pollen are unlikely to be caused by beetle attacks.

Daily average pollen concentrations are expressed as particles per cubic metre of air (P m^{-3}) (Comtois 1998). The amount of pollen recorded during August and September (AAP, ACP and AUP) is presented as grains.

2.2 Meteorological data

Meteorological data were collected at Malpensa airport and accessed via http://www.tutiempo.net. The following daily meteorological variables were examined: T, mean temperature (°C); T_{max}, maximum temperature (°C); T_{min}, minimum temperature (°C); H, mean humidity (%); PP, precipitation amount (mm); V, mean wind speed (Km/h); VM, maximum sustained wind speed (Km/h); RN, indicator for occurrence of rain or drizzle; SN, indicator for occurrence of snow or ice Pellets; FG, indicator for occurrence of fog. Meteorological data for Malpensa airport were not available for analysis: 2002—26–31.05.2002; 2002—18–21.06.2002; 2005—13–30.11.2005; 2007—28.8.2007; 2008—18.8.2008; 2013—1–31.12.2013. In addition, daily average wind direction data were obtained from Busto Arsizio meteorological station, which is situated close to Legnano (ITMIL6) pollen-monitoring station and the meteorological station at Malpensa airport (Fig. 1).

2.3 Statistical analysis

Data were examined for normality using the Kolmogorov–Smirnov test. Where data were found to be normally distributed, parametric statistics were applied; where data were not normally distributed, nonparametric statistics were used (Sterling et al. 1999).

Annual variations in the amount of airborne pollen recorded during August and September were normally distributed. Analysis of variance (ANOVA) was used to determine whether there was a significant difference in the mean AAP recorded at the three sites (2000–2013). Also, Pearson's correlation analysis was carried out between AAP recorded at all three sites included in the study, as well as between AAP, ACP and AUP recorded at Legnano. Trends in the amount of *Ambrosia*, Cannabaceae and Urticaceae pollen recorded in three periods (2000–2011, 2000–2012 and 2000–2013) were examined using simple linear regression analysis. The following statistics are presented: the slope of the simple linear regression over time, standard error of the regression slope (SE) and probability level (p).

Conversely, daily variations in airborne pollen and variations in meteorological data (daily and monthly) were often not normally distributed. As a result, Spearman's rank correlation analysis was used to examine relationships between the following: (1) daily average concentrations of airborne *Ambrosia* pollen with daily average concentrations of airborne Cannabaceae and Urticaceae pollen; (2) daily average concentrations of airborne *Ambrosia*, Cannabaceae and Urticaceae pollen with all of the daily meteorological data listed in Sect. 2.2 (not wind direction); (3) AAP with monthly meteorological data recorded from January through to September in the year of pollination. The latter was used to determine whether there was a relationship between the amount of airborne *Ambrosia* pollen recorded in August and September and meteorological variables likely to influence the production, release and dispersal of *Ambrosia* pollen. The monthly meteorological data were as follows: monthly averages of daily temperature (daily minimum, mean and maximum temperatures), daily average relative humidity and daily precipitation; the monthly sum of daily precipitation; the number of days with rain, fog and drizzle recorded in each month. In addition, two monthly sums and averages of precipitation, humidity and temperature were also entered into correlation analysis with AAP. Data from 2013 were not entered into Spearman's rank correlation analysis, as this was the year under investigation (2000–2012 only).

Simple linear and multiple linear regression models were constructed in order to predict AAP in "below average", "above average" and "low intensity" years (Sect. 2.4) recorded at Legnano, which is the site closest to the meteorological station at Malpensa airport (Fig. 1). The justification for this extra analysis is that if the models were unable to satisfactorily predict the AAP in 2013, then this would add weight to the argument that the observed decrease in airborne concentrations of *Ambrosia* pollen cannot be explained by meteorology and add support to the hypothesis that the decrease was related to the presence of *O. communa* in the area. Independent variables that were not normally distributed were normalised using the LOG10 function (Stach et al. 2008). Spearman's correlation analysis was then repeated, in order to ensure that normalisation had not notably altered the strength and direction of the relationship between dependent and independent variables.

Tests were considered significant with probability levels <0.05. All calculations and statistical analysis of the data were conducted using Microsoft Excel and SPSS 12.0 software packages.

2.4 Model for predicting annual variations in Ambrosia pollen (AAP)

The independent variables with the strongest relationship with AAP were entered into simple linear and multiple linear regression analysis. The regression models were primarily constructed in order to investigate whether the AAP in 2013 was lower than would normally be expected based on the prevailing weather conditions. The regression models were also validated using data deemed to be characteristic of "below average" (2010) and "above average" (2009) magnitude AAP years (note that 2013 recorded the lowest AAP in the 2000–2013 data set and was therefore deemed to be a "low intensity" year).

The regression models were calibrated using 11 years of *Ambrosia* pollen and environmental data (2000–2008, 2011 and 2012). These years were not included in the validation process. The models were validated (tested) using 2 years of data not included in model construction. Following the method described by Prentović et al. (2014), it was decided not to test the performance of the model using the two most recent years of data (i.e. 2011 and 2012). This was because both of these years were representative of low AAP years. Instead, it was decided to validate the model using data that were characteristic of both low and high seasons (but not the years with the lowest and highest AAP in the data set). For this reason, the AAP in each year was categorised as being "below average" or "above average". The years with the mean AAP in each group (e.g. the mean of all "below average" years) were selected for validation. The 2 years selected were 2010 (mean of all "below average" years) and 2009 (mean of all "above average" years). The regression models were then validated using the AAP from selected validation years (2009 and 2010), as well as 2013.

The models were evaluated by examining: (a) the adjusted coefficient of determination (Adj.R^2), which is considered more appropriate for evaluating regression models developed on small samples (Pallant 2001); (b) the Kolmogorov–Smirnov test (normality of the residuals); (c) the Koenker–Basset test for heteroscedasticity, for violation of homogeneity of the residuals variance (Bickel 2012); (d) collinearity diagnostics were carried out by checking the variance inflation factor (VIF) and computing the Mahalanobis distance (Pallant 2001).

2.5 Examining land use change in the Milan area

Common ragweed is a pioneer plant that is known to prefer open, disturbed sites (Essl et al. 2009) and tends to grow in suitable habitats if two conditions are fulfilled (Skjøth et al. 2010): (1) available seeds; (2) soil disturbance. For this reason, major construction works in the Milan area 2000–2013 were examined in order to determine whether changes in land use could be related to the decrease in airborne concentrations of *Ambrosia* pollen in 2013. Information about construction sites were accessed via the Local Health Authority Milan 1, Department of Medical Prevention, UOC SPreSAL, Internal Archive. The "UOC SPreSAL" is the service that controls construction in the Milan area. Geographical coordinates were found by using Google Maps.

3 Results

3.1 Trends and relationships in the amount of airborne pollen

There is great deal of variation in AAP recorded at stations situated in the north-west of the Province of

Milan (Table 1). The highest AAP was recorded in 2000, 2004 and 2009 in the Milan area (the highest was 2000 at Legnano and Rho and 2004 at Magenta). The lowest AAP was recorded in 2013 at all three sites. Note that the second lowest AAP was recorded in 2012, showing a notable decrease in recent years. The results of ANOVA showed that there was not a significant difference ($p > 0.05$) between the AAP recorded at the three sites, and Pearson's correlation analysis showed that AAP at these sites was also significantly (positively) related. The amount of airborne Urticaceae pollen recorded annually showed a similar pattern, with the highest AUP in 2000 and lowest AUP in 2013. The lowest amount of airborne Cannabaceae pollen was also recorded in 2013, but the highest ACP was in 2009.

There were a number of significant negative trends in AAP during the period 2000–2013 (Table 2). These significant trends in AAP were no longer evident when *Ambrosia* pollen data from 2013 and 2012 were removed from the analysis. The only site where there were no significant trends in AAP was Magenta, where the peak AAP was recorded in 2004. The significant negative trend was still apparent when data from all three sites were combined. There were no significant trends in ACP, but there were significant negative trends in AUP in all three periods (2000–2013, 2000–2012 and 2000–2011).

Cannabaceae and Urticaceae plants also flower in late summer, and their pollen is in the air at the same time as *Ambrosia*. The results of Spearman's correlation analysis showed that there were significant positive relationships between daily average concentrations of airborne *Ambrosia* pollen and airborne Cannabaceae pollen ($r = 0.5582$, $p < 0.001$) and Urticaceae pollen ($r = 0.648$, $p < 0.001$) recorded during August and September at the sites. There was also a significant positive correlation between daily average concentrations of airborne Cannabaceae pollen and airborne Urticaceae pollen ($r = 0.714$, $p < 0.001$).

Pearson's correlation analysis showed that there was a significant relationship between AAP and ACP ($r = 0.560$, $p = 0.037$) but not between AAP and AUP ($r = 0.490$, $p = 0.075$) recorded at Legnano. Similarly, correlations were not significant between ACP and AUP at this site ($r = 0.176$, $p = 0.547$).

3.2 The influence of meteorological data on airborne pollen concentrations

Daily average concentrations of airborne *Ambrosia*, Cannabaceae and Urticaceae pollen were entered in Spearman's correlation analysis with daily meteorological data (2000–2013). The results show that there were a number of significant correlations between daily average concentrations of airborne pollen and daily meteorological data recorded during August and September (Table 3). Daily average pollen concentrations were negatively correlated with precipitation and humidity and positively correlated with temperature. The only significant correlations with wind speed were seen with airborne Urticaceae pollen at Legnano (significant negative correlations). Wind direction was also examined. Wind roses (Fig. 2a–f) for daily average wind direction during August and September show that there was a higher than average frequency of winds from the Southeast in 2012 and 2013 (the years with the lowest AAP). Conversely, there was a higher frequency of winds from the South and Southwest in 2000 and 2004 (years with high AAP), although it should be noted that there was also a high frequency of Southeasterly winds in 2009 when the AAP also exceeded 6000 *Ambrosia* pollen grains (Fig. 2c; Table 1).

AAP recorded in Legnano was entered into Spearman's rank correlation analysis with monthly and two monthly averages of meteorological data recorded before August (examining pollen production) and during August and September (related to the release and dispersal of pollen from the plant) in the year of pollination. The only statistically significant correlations recorded were between AAP and the following (Fig. 3):

- Before the season—March ($r = -0.702$, $p = 0.008$) and June ($r = -0.634$, $p = 0.020$) averages of daily minimum temperatures, as well as June averages of daily relative humidity ($r = -0.676$, $p = 0.011$);
- During the season—September number of days with rain ($r = -0.656$, $p = 0.015$).

Climate data recorded at Malpensa airport (Table 4) show that in 2013 mean minimum temperatures in March and June, mean relative humidity in June and the number of days with rain in September 2013 were all below average (lower than the 1981–2010 mean).

Table 1 Amount of *Ambrosia* (AAP), Cannabaceae (ACP) and Urticaceae (AUP) pollen recorded annually during August–September from 2000 to 2013 at individual aerobiological stations situated in the Northwest of the Province of Milan: Legnano (ITMIL6), Magenta (ITMAGE) and Rho (ITRHO1)

Year	*Ambrosia* ITMIL6		*Ambrosia* ITMAGE		*Ambrosia* ITRHO1		*Ambrosia* Milan area (ITMAGE, ITMIL6, ITRHO1 mean)		Cannabaceae ITMIL6		Urticaceae ITMIL6	
	AAP	Maximum	AAP	Maximum	AAP	Maximum	AAP	Maximum	ACP	Maximum	AUP	Maximum
2000	7062	447	6753	370	7442	528	7086	394	1089	88	7574	463
2001	4872	461	3795	257	3147	247	3938	322	815	55	5494	413
2002	3545	310	5371	683	5053	585	4656	526	618	43	7428	376
2003	4928	292	5999	655	4476	208	5134	296	990	88	2155	132
2004	6232	337	7358	497	5585	452	6392	380	757	75	2982	167
2005	4467	433	5728	583	4623	273	4939	380	1144	97	3954	391
2006	5365	737	5042	461	6421	522	5609	427	758	73	4255	356
2007	4837	288	7040	1125	5593	527	5823	630	758	93	2711	206
2008	3942	255	6511	400	4146	241	4866	258	927	54	4814	304
2009	6162	308	7212	331	5424	324	6266	281	1350	126	4597	254
2010	3319	296	6570	767	3610	409	4500	329	540	32	3078	223
2011	3922	253	6753	1001	3659	256	4778	387	1264	105	2735	141
2012	2791	194	3603	413	3013	191	3136	220	820	86	1483	63
2013	**646**	48	**606**	65	**803**	84	**685**	48	**449**	34	**1447**	134
Mean	4435.00	332.79	5595.79	543.43	4499.64	346.21	4843.43	348.43	877.07	74.93	3907.64	258.79
SD	1621.43	157.35	1858.82	286.05	1646.14	155.82	1575.67	137.52	265.83	27.97	1944.00	125.44

The lowest value is written bold, and the highest value is underlined

Table 2 Trends in the amount of airborne *Ambrosia* (AAP), Cannabaceae (ACP) and Urticaceae (AUP) pollen recorded annually in August–September in the Milan area during three periods (2000–2011, 2000–2012 and 2000–2013): slope of the regression over time (slope), standard error of the regression (SE) and probability level (*p*). Legnano (ITMIL6), Magenta (ITMAGE) and Rho (ITRHO1)

Site	2000–2013			2000–2012			2000–2011		
	Slope	SE	*p*	Slope	SE	*p*	Slope	SE	*p*
AAP—ITMIL6	−256.312	83.932	**0.01**	−174.659	81.091	0.054	−134.318	91.905	0.175
AAP—ITMAGE	−136.200	122.096	0.286	21.665	94.871	0.824	135.601	80.886	0.125
AAP—ITRHO1	−240.556	89.897	**0.02**	−158.516	89.223	0.103	−121.248	102.455	0.264
AAP—Milan area	−211.023	90.065	**0.037**	−103.837	76.415	0.201	−39.988	79.200	0.625
ACP—ITMIL6	−0.069	18.225	0.699	7.434	18.849	0.701	13.552	21.907	0.550
AUP—ITMIL6	−330.631	94.266	**0.004**	−318.648	109.782	**0.014**	−286.738	128.054	**0.049**

Bold values are signify the probability level

Table 3 Results of Spearman's rank correlation analysis between daily average *Ambrosia*, Cannabaceae and Urticaceae pollen concentrations (August–September) and daily meteorological data. *T* mean temperature (°C), T_{max} maximum temperature (°C), T_{min} minimum temperature (°C), *H* mean humidity (%), PP precipitation amount (mm), *V* mean wind speed (Km/h), VM maximum sustained wind speed (Km/h)

	Ambrosia Milan area (ITMAGE, ITMIL6, ITRHO1 mean)	Cannabaceae ITMIL6	Urticaceae ITMIL6
PP	−0.093**	−0.183**	−0.164**
H	−0.140**	−0.256**	−0.125**
T_{min}	0.119**	0.439**	0.351**
T_{max}	0.176**	0.556**	0.429**
T	0.184**	0.567**	0.470**
V	−0.008	0.030	−0.124**
VM	−0.024	0.000	−0.135**

* Correlation is significant at the 0.05 level (two tailed)

** Correlation is significant at the 0.01 level (two tailed)

3.3 Regression analysis

Simple linear and standard multiple regression models were constructed for predicting the AAP in Legnano during 2009 and 2010 (the validation years) as well as 2013 (the year under investigation), using meteorology parameters that were significantly correlated with AAP. The final models are presented in Table 5. The only models that were statistically significant were the simple linear regression model using the number of days with precipitation in September and standard multiple regression models using: (1) March averages of daily minimum temperature and the number of days with precipitation in September and (2) June averages of daily relative humidity and the number of days with precipitation in September. In general, actual and predicted values for AAP were reasonably close in both 2009 and 2010. The main exception is the simple linear regression model that included the number of days with precipitation in September, which overestimated AAP in 2010. There was a close agreement between the models in 2013, but all three models greatly overestimated AAP in this year (Fig. 4).

3.4 Land use change

Examination of data from the UOC SPreSAL Internal Archive shows that there is no relationship between the number of large construction works (i.e. the Milan Fair, road and rail networks and building construction such as hospitals and residential buildings) and the significant decrease witnessed in AAP in the Milan area since 2000 (Table 6). No major construction works were listed as being undertaken during 2000 and 2001, when airborne concentrations of *Ambrosia* pollen were high. On the other hand, a number of major construction works have been in operation in recent years, such as residential building as well as road and rail links.

4 Discussion

This study investigates potential drivers of the significant decrease witnessed in *Ambrosia* pollen counts recorded in the Milan area in recent years.

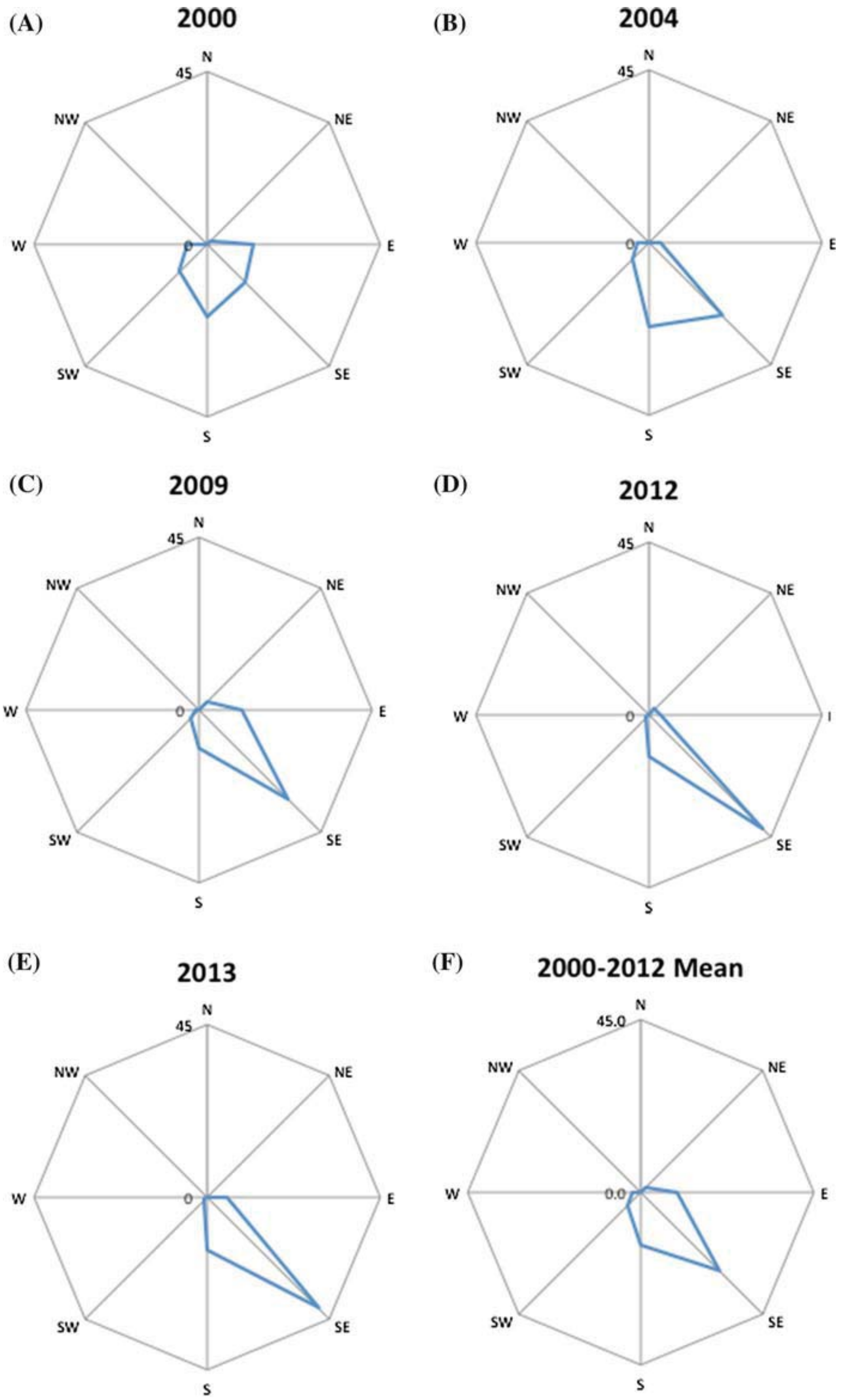

Fig. 2 Wind Roses for selected years, showing the frequency different daily average wind directions recorded at Busto Arsizio meteorological station during August and September in 2000–2013: **a** 2000; **b** 2004; **c** 2009; **d** 2012; **e** 2013; **f** 2000–2012 mean

Concentrations of airborne *Ambrosia* pollen recorded at the three pollen-monitoring sites included in this study are closely related (determined by ANOVA and Pearson's correlation analysis). This information, combined with the fact that this significant decrease is repeated at all three sites, suggests that this is a

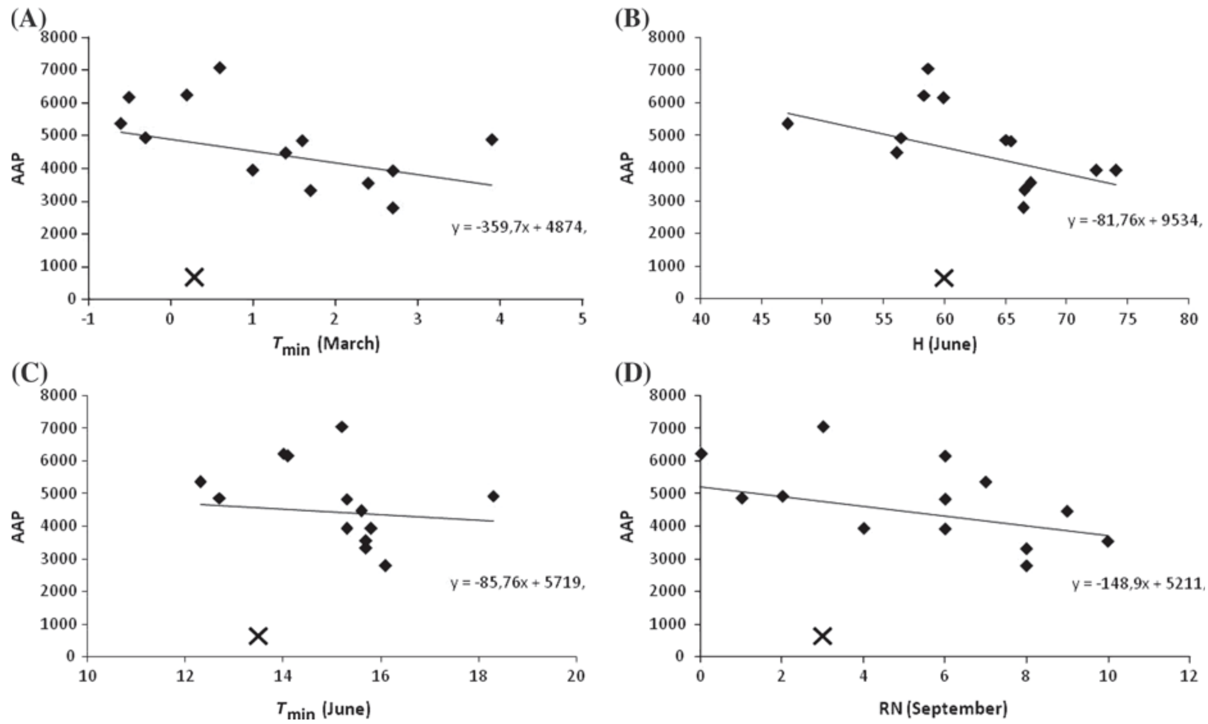

Fig. 3 Relationship between AAP and monthly average meteorological data with significant correlations: **a** March averages of daily minimum temperature, **b** June averages of daily relative humidity, **c** June averages of daily minimum temperatures, (*D*) number of days with rain in September. Data from 2013 are marked as "*X*". Note that the negative relationship with the monthly variables means that low values would normally result in high AAP. Many of the values are below average (e.g. T_{min} in June is the third lowest in the 2000–2013 data set), but the AAP in 2013 is extremely low. T_{min} minimum temperature (°C), *H* mean humidity (%), RN indicator for occurrence of rain or drizzle

Table 4 Climate data recorded at Malpensa airport and accessed via http://www.tutiempo.net, showing 2013 values in relation to long-term mean for the period 1981–2010

	2013	1981–2010
Mean annual T_{min}	6.50	5.93
Mean annual RN	150.00	127.14
T_{min} (March)	0.30	0.58
T_{min} (June)	13.50	13.60
H (June)	60.00	67.64
RN (September)	7.00	10.50

T_{min} minimum temperature (°C), *H* mean humidity (%), RN indicator for occurrence of rain or drizzle

regional scale phenomenon. The question that this study has attempted to answer is: Can this decrease be explained by environmental factors such as meteorology, or is there evidence to support the hypothesis that the decrease was related to the presence of large numbers of *O. communa* leaf beetles in the area?

Airborne concentrations of *Ambrosia* pollen recorded in the Milan area are influenced by weather conditions before and during the main flowering period of *Ambrosia* (August–September). The weather before the start of the season influences pollen production, and significant correlations were found between AAP and March and June averages of daily minimum temperatures, as well as June averages of daily relative humidity. The negative relationships between AAP and these variables suggest that lower temperatures in March and lower temperatures and lower rainfall in June favour the production of *Ambrosia* pollen. It should therefore be noted that minimum temperatures in March and June as well as relative humidity in June were all below average in 2013 (compared with the 1981–2010 mean) (Table 4).

The negative association with minimum temperatures in March could be caused by the relationship

Table 5 Simple linear and standard multiple regression models for predicting AAP for Legnano

Model no.	Regression equation	Adj. R^2	p	Kolmogorov–Smirnov test (p)[a]	Koenker–Basset test (p)[b]
1	5392.861-471.691*T_{min} (March)	0.222	0.081	0.172	0.876
2	8263.214-234.109*T_{min} (June)	0.002	0.339	0.200	0.410
3	10,090.968-85.973*H (June)	0.241	0.072	0.200	0.337
4	5860.751 + 223.308*RN (September)	0.308	**0.045**	0.200	0.252
5	9746.913-510.249*T_{min} (March)–248.385*T_{min} (June)	0.310	0.093	0.200	0.592
6	8460.430-262.836*T_{min} (March)–53.883*H (June)	0.207	0.162	0.200	0.551
7	6234.579-380.312*T_{min} (March)-190.795*RN (September)	0.455	**0.036**	0.200	0.728
8	8473.372-176.775*T_{min} (June)–211.543* RN (September)	0.292	0.103	0.200	0.792
9	10,751.921-79.487*H (June)–209.352*RN (September)	0.558	**0.016**	0.200	0.685

Models calibrated using data not included in model validation. T_{min} (March) March averages of daily minimum temperatures (°C), T_{min} (June) June averages of daily minimum temperatures (°C), H (June) June averages of daily relative humidity (%), RN (September) number of days with precipitation (rain or drizzle) in September

Bold values are signify the probability level

[a] $p > 0.05$ normality of residuals distribution is not violated

[b] $p > 0.05$ homogeneity of residual variance (homoscedasticity) is not violated

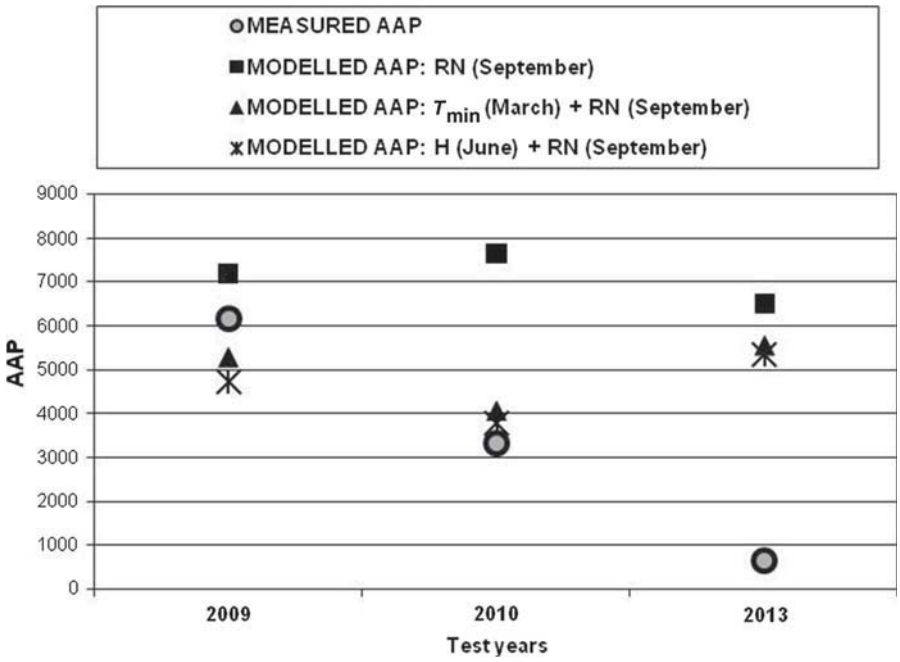

Fig. 4 Performance of significant regression models in forecasting AAP in Legnano during mean "above average" (2009) and mean "below average" (2010) years, as well as AAP in 2013 (the year with the lowest AAP in the 2000–2013 dataset). T_{min} minimum temperature (°C), H mean humidity (%), RN indicator for occurrence of rain or drizzle

between germination success and the size of the source (i.e. the number of plants in the region). This is because *Ambrosia* seeds require moist chilling (stratification) before they can germinate (Pickett and Baskin 1973; Willemsen 1975; Baskin and Baskin 1977, 1980). *Ambrosia* plants that emerge earlier have been shown to grow larger, and *Ambrosia* pollen production is directly correlated with biomass (Rogers et al. 2006; Fumanal et al. 2007). In addition, lower temperatures in June may facilitate reproductive growth because, as seen with chrysanthemums (close relatives to both *Ambrosia*), exposure to high temperatures at the beginning of the short-day period results in a notable delay both in initiation and further

Table 6 Major construction works in the Milan area 2000–2013

Construction works	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Geographical coordinates
Milan Fair (located in Rho-Pero) from 2002 to 2005			■	■	■	■									45.520796, 9.078340
Three other large roadworks located in Rho-Pero from 20 02 to 2005			■	■	■	■									45.520796, 9.078340 and the surroundings
TAV (TGV) rail line from 2005 to 2010						■	■	■	■	■	■				From 45.472386, 8.819784 to 45.513395, 9.051996
Expo 2015 (near Milan Fair Rho-Pero) from 2012 – in progress													■	■	45.520796, 9.078340
Boffalora-Malpensa highway (road link between S.S n. 527 and S.S. n. 11) from September 2004 to March 2008					■	■	■								From 45.467913, 8.862821 to 45.588893, 8.779394
FNM (rail link and doubling of the railway line) from June 2006 to January 2009							■	■	■						45.577670, 8.787510
FNM (upgrading of the railway line) from May 2011 – in progress												■	■	■	From 45.577670, 8.787510 to 45.528013, 8.738758
Adaptation and modernisation of A4 highway from January 2012 – in progress													■	■	From 45.473571, 8.816284 to 45.490377, 8.865858
Garbagnate General Hospital from July 2011 – in progress												■	■	■	45.582718, 9.097313
Legnano General Hospital from January 2007 to May 2010								■	■	■					45.583940, 8.886753
D4 (residential building construction site situated in Assago) from January 2007 – in progress								■	■	■	■	■	■	■	45.406347, 9.123984
Doubling of the railway line Milano-Mortara from February 2007 to 2010								■	■	■					From 45.420613, 9.066950 to 45.432456, 9.109696

Information was accessed via the Local Health Authority Milan 1, UOC SPreSAL, Internal Archive. The “UOC SPreSAL” is the service that controls construction works in the Milan area. Geographical coordinates were found by using Google Maps. Shaded areas show when large construction works were undertaken

development of the terminal flowers (Cockshull and Kofranek 1994). The authors have not been able to identify a causal mechanism that can satisfactorily explain why low relative humidity in June would be beneficial for the production of pollen by *Ambrosia* plants.

Interestingly, there is evidence to suggest that higher temperatures and humidity can aid the reproduction and survival rates of *O. communa* leaf beetles. It has been shown that increased temperatures shorten the preovipositional period, ovipositional period and longevity of female *O. communa* with the highest amount of oviposition observed at 28 °C. Survival rates of *O. communa* from egg-to-adult stages were also much higher at 25 and 28 °C (Zhou et al. 2010b). Higher relative humidity also improves survival rates during the egg, larva and entire immature stage of *O. communa* (Zhou et al. 2010a). However, it is suspected that *O. communa* has been present in Europe for less than 5 years (Müller-Schärer et al. 2014), and so it is debatable whether this would be long enough to influence the long-term relationship (2000–2012) between airborne concentrations of *Ambrosia* pollen and weather conditions in spring and summer.

This study did not simply examine relationships between airborne *Ambrosia* pollen concentrations and meteorological factors. Regression models were constructed in order to predict the AAP in “below average”, “above average” and “low intensity” years. The idea is that failure of the models to satisfactorily predict the AAP in 2013 would corroborate the evidence showing that the observed decrease in airborne concentrations of *Ambrosia* pollen cannot be explained by meteorology add that the decrease was related to the presence of *O. communa* in the area. The regression models tended to overestimate the amount of *Ambrosia* pollen recorded in August and September 2013, which indicates that the AAP in this year was much lower than expected. For instance, examination of the relationship between AAP and monthly meteorological data in 2013 (Fig. 3) shows that low minimum temperatures in March, low minimum temperatures and relative humidity in June, and a low number of days with rain in September ought to have resulted in AAP being much higher. This evidence is not conclusive, however, because it should also be remembered that regressions are used to predict the future by examining the past and as such have difficulty modelling extreme values outside the range of data used to construct them.

As seen with airborne *Ambrosia* pollen, there was a significant decrease in the amount of Urticaceae pollen recorded over the period 2000–2013. In addition, like *Ambrosia*, the amounts of atmospheric Urticaceae pollen recorded in 2012 and 2013 were lower than in other years in the data set. The lowest amount of

Cannabaceae pollen was also recorded in 2013. The amount of Cannabaceae and Urticaceae pollen recorded in August and September cannot, therefore, be used to support the hypothesis that meteorology did not play a part in the decrease in airborne concentrations of *Ambrosia* pollen (i.e. it is possible that inclement—wet—weather in August and September 2013 caused *Ambrosia*, Cannabaceae and Urticaceae pollen concentrations to decrease).

Daily average *Ambrosia* pollen concentrations in the Milan area were found to be negatively related to daily precipitation, and AAP was negatively correlated with the number of days with precipitation in September (during the main flowering period of *Ambrosia*). This is related to the release and dispersal of pollen, because precipitation removes bioaerosols from the air (Cox and Wathes 1995). With this in mind, it should be noted that the number of days with precipitation in September 2013 was below average (lower than the 1981–2010 mean) (Table 4). The results of correlation analysis also showed that there are significant positive relationships between daily average concentrations of airborne *Ambrosia*, Cannabaceae and Urticaceae pollen, which suggests that similar factors, such as meteorology, influence daily variations in the amount of pollen recorded in the air during August and September for all three pollen types. It is interesting to note, therefore, that 2013 was a relatively dry year. August and September 2013 recorded in the third and fifth lowest mean monthly rainfall in the 2000–2013 data set, respectively (data not shown).

It is conceivable that changes in land use could influence airborne concentrations of *Ambrosia* pollen. *Ambrosia* has a preference for disturbed sites (Essl et al. 2009), but examination of information from the UOC SPreSAL Internal Archive shows that there is no relationship between construction works and the decrease in atmospheric *Ambrosia* pollen in the Milan area (Table 6). The implementation of management programmes designed to reduce *Ambrosia* populations (Ordinanza_Del_Presidente_Della_Regione_Lombardia 1999; Regione_Lombardia 2013) could also be responsible for the reduction in atmospheric concentrations *Ambrosia* pollen, as well as airborne pollen from other plants growing on similar habitats. It is therefore important to note that the regional instructions for *Ambrosia* containment specify that the plant should be controlled in both ruderal and agricultural habitats, and so management could partly explain the decreases seen in *Ambrosia*, Cannabaceae and Urticaceae pollen concentrations.

5 Conclusions

The results of this study do not reject the hypothesis that the observed decrease in airborne *Ambrosia* pollen was mainly due to the presence of large numbers of *O. communa* in the Milan area. There is some evidence to suggest that, based on weather conditions in 2013, airborne *Ambrosia* pollen concentrations should have been higher than recorded and that the drastic decrease in AAP in 2013 cannot be explained by meteorology alone. It is necessary to determine to what degree the presence of *O. communa* will benefit the allergic population, and so studies are presently underway that will further assess the effects of the ragweed leaf beetle infestations on local pollen production and local and regional concentrations of airborne *Ambrosia* pollen.

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