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Title

POLLEN CONCENTRATIONS AND PREVALENCE OF ASTHMA AND ALLERGIC RHINITIS IN ITALY: EVIDENCE FROM THE GEIRD STUDY.

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ABSTRACT

Background: Pollen exposure has acute adverse effects on sensitized individuals. Information on the prevalence of respiratory diseases in areas with different pollen concentrations is scanty.

Aim: We performed an ecologic analysis to assess whether the prevalence of allergic rhinitis and asthma in young adults varied across areas with different pollen concentrations in Italy.

Methods: A questionnaire on respiratory diseases was delivered to random samples of 20-44 year-old subjects from six centers in 2005-2010. Data on the daily air concentrations of 7 major allergologic pollens (*Poaceae, Urticaceae, Oleaceae, Cupressaceae, Coryloideae*, *Betula* and *Ambrosia*) were collected for 2007-2008. Center-specific pollen exposure indicators were calculated, including the average number of days per year with pollens above the low or high concentration thresholds defined by the Italian Association of Aerobiology. Associations between pollen exposure and disease prevalence, adjusted for potential confounders, were estimated using logistic regression models with center as a random-intercept.

Results: Overall, 8834 subjects (56.8%) filled in the questionnaire. Allergic rhinitis was significantly less frequent in the centers with longer periods with high concentrations of at least one (OR per 10 days =0.989, 95%CI: 0.979-0.999) or at least two pollens (OR=0.974, 95%CI: 0.951-0.998); associations with the number of days with at least one (OR=0.988, 95%CI: 0.972-1.004) or at least two (OR=0.985, 95%CI: 0.970-1.001) pollens above the low thresholds were borderline significant. Asthma prevalence was not associated with pollen concentrations.

Conclusions: Our study does not support that the prevalence of allergic rhinitis and asthma is greater in centers with higher pollen concentrations. It is not clear whether the observed ecologic associations hold at the individual level.

Highlights

- The chronic effects of pollens on allergic disease prevalence are poorly known
- We calculated center-specific indicators of long-term pollen exposure
- We studied if pollen levels are related to allergic rhinitis and asthma in Italy
- Allergic rhinitis was less frequent in centers with higher pollen loads
- There was no ecologic association of pollen exposure with asthma prevalence

Keywords (max 6)

Aeroallergen, adult, allergy, ecologic study, public health, respiratory.

INTRODUCTION

Asthma and rhinitis are allergic respiratory diseases affecting, respectively, 5-15% and 20-30% of adults in Europe (Bauchau and Durham, 2004; Jarvis et al. 2012). The prevalence of both diseases shows great heterogeneity both between and within countries (Burney et al., 1994; Björnsson et al., 1994). Geo-climatic factors seem to account for part of this variability (Pesce et al. 2016). Climatic and meteorological conditions influence the regional flora and intensity of pollination, the dispersion of pollens and the duration of the pollen season (Latorre and Caccavari,2009, Mandrioli and Negrini in D'Amato,1991). Climate change may be in part responsible for the observed increases in asthma and allergic rhinitis prevalence in the last decade (Zanolin et al., 2004, de Marco et al. 2012), possibly through its effects of altering pollination (De Sario et al., 2013; D'Amato at al., 2014).

Exposure to pollens and other aeroallergens may cause allergy, which is a hyperreactivity reaction of the respiratory tract and eye conjunctiva mediated by immunological (mostly IgE-dependent) inflammation. In sensitized individuals, exposure to pollens leads to release of bioactive molecules such as histamine and lipid mediators that can cause smooth muscle contraction, increased vascular permeability, mucus secretion, and attract inflammatory cells (Plotz et al. 2004, Bernstein et al. 2016).

Pollens are one of several environmental factors that trigger symptoms of respiratory allergic diseases, along with meteorological conditions (extreme temperatures, thunderstorms) and chemical air pollutants (D'Amato et al. 2000; Celenza et al. 1996). Nonetheless few studies have investigated whether the prevalence of respiratory diseases varies across areas with different airborne pollen concentrations. All published studies investigated children (Silverberg et al. 2015; Burr et al., 2003; Yoshida et al., 2013). Inconsistent results from these studies showed either positive, null or negative associations between pollen concentrations and respiratory diseases, which may partly relate to the different populations and age groups examined, as well as to the different vegetation and climatic conditions of the geographical areas.

The aim of this ecologic study is to describe the cross-sectional relationship between annual concentrations of major allergologic pollens and the prevalence of allergic rhinitis and asthma in the Italian centers participating in the GEIRD (Gene Environment Interactions in Respiratory Diseases) study.

METHODS

Study design

The phase one of the GEIRD multicenter study had a cross sectional design. Three thousands subjects of the Italian young adult general population aged 20-44 years (male to female ratio equal to 1) were selected from six centers between 2005 and 2010 (de Marco et al., 2010). Three centers (Turin, Pavia and Verona), in northern Italy, have a subcontinental climate (Köppen classification: CFA). The other three centers (Ancona, Sassari and Salerno) are coastal cities in central, insular and southern Italy respectively, and they are characterized by a mediterranean climate (Köppen classification: CSA). The subcontinental zone has a lower annual average temperature and a higher annual temperature range than the mediterranean zone (Pesce et al. 2016).

Questionnaire and outcomes

A brief postal questionnaire was self-administered for up to three times. In the case of final non response, a phone interview was carried out. The questionnaire (available at www.geird.org) investigated respiratory symptoms and diseases, socio-economic factors, smoking habits and exposure to vehicular traffic. The questions were derived from the European Community Respiratory Health Survey (de Marco et al., 1999). Each center obtained approval from the Ethics committee. All participants were fully informed about all aspects of the research project and consented to take part in the survey. A subject was considered to have:

- ***Allergic rhinitis*** if he/she answered “yes” to the question: “Do you have any nasal allergies including hay fever?”.
- ***Asthma*** if he/she answered “yes” to both questions: ‘‘Have you ever had asthma?’’ and ‘‘Was this confirmed by a doctor?’’.

Pollen data

Data on the daily air concentrations of seven pollens from five vegetal families (*Poaceae, Urticaceae, Oleaceae, Cupressaceae*), one subfamily (*Coryloideae*), and two genera (*Betula* and *Ambrosia*) were collected from local monitoring stations. The monitoring stations were within city centers, with the exception of Salerno, which had no local monitoring station. In this case we used data from the closest station (Napoli Portici) to derive pollen indicators (see graphical abstract, left panel). All the monitoring stations used a volumetric sampler of the Hirst type. The device consists of a pump which operates continuously for seven days and is calibrated to aspirate 10 l/min of air (14.4 m^3) in 24h. The air is directed on a sampling surface constituted by a metallic drum which rotates at the speed of 2 mm/h, on which a plastic adhesive tape of silicone oil retains airborne particles. After seven days, the sampling drum with the adherent atmospheric particles is cut into fragments corresponding to the monitoring days. Each fragment is placed on a slide and covered with glycerin jelly mixed with basic fuchsine (Odgen et al. 1974) and examined under a microscope at 400x magnification. The daily pollen concentration is determined as the number of pollen grains per cube meter of air (grains/m^3).

Missing data in the time-series of pollen concentrations were considered to be non-randomly allocated over time, since they could be generated by temporary instrumental breakdowns and maintenance. For this reason we decided to input them using the k-Nearest Neighbor algorithm (Fix and Hodges, 1951; Dixon, 1979). This algorithm assigns missing values based on their similarity to k non-missing values according to a Euclidean distance function. In our data, we set k=9 and we implemented the similarity function accounting for year, month, day, and calendar day.

Ecologic indicators of pollen exposure

For each pollen taxa and for each center, we calculated the number of days, over a calendar year, when pollen concentrations were above the low and high concentration thresholds defined by the Italian Aerobiological Monitoring Network (*Rete Italiana di Monitoraggio in Aerobiologia*) of the Italian Association of Aerobiology (*Associazione Italiana di Aerobiologia*) [http://www.ilpolline.it/]. Low and high thresholds in grains/m^3 are: *Poaceae,* 0.6 and 30; *Urticaceae,* 2 and 70; *Oleaceae,* 0.6 and 25; *Cupressaceae,* 4 and 90; *Coryloideae*and *Betula,* 0.6 and 50; and *Ambrosia,* 0.1 and 25. We also computed the number of days, over a calendar year, when the concentrations of *at least one* and *at least two pollens* were above the low and high thresholds, respectively. Finally, we calculated “pollen intensity” as the sum of daily concentrations over a calendar year.

In the main analysis, center-specific proxy indicators of long-term pollen exposure were obtained by averaging the previously mentioned indicators over years 2007 and 2008, which is an intermediate period during questionnaire data collection. However, under the hypothesis that self-reporting of allergic diseases may be influenced by the level of symptoms at the time of participation, alternative exposure indicators were calculated using daily data from the years of questionnaire administration in each center (ranging from 2005-2006 in Ancona to 2009-2010 in Turin, see supplementary figure S1) and used for sensitivity analysis.

Statistical analysis

. The associations (OR with 95% Confidence Interval, 95%CI) between the pollen exposure indicators and the prevalence of allergic rhinitis and asthma were assessed using two-level logistic regression models, with center as a random intercept. All the analyses were adjusted for gender, age (mean centered), smoking habits (non-smokers, ex-smokers, smokers with <15 cigarette pack-years, and smokers with ≥15 pack-years), education level (lower school, high school, degree), heavy traffic near home (never/rare, frequent/constant), and climatic zone (subcontinental, mediterranean). To improve the comparability across study centers, we also adjusted for the following design variables: season of response, type of contact for questionnaire administration (mail, phone), percentile rank of response (mean centered). The latter was included to account for differences in response rates between centers [de Marco et al., 1994] The analysis on asthma was also adjusted for the presence of allergic rhinitis.

RESULTS

Overall, 8834 subjects filled in the questionnaire with a 56.8% of response (range: 37.1% Pavia - 67.7% Verona), 47.5% of them were males, and the median age was 35.2 years (Interquartile range: 11.8 years). Subjects from the mediterranean zone were more likely to be males, young, smokers and exposed to high traffic levels than subjects from the subcontinental zone (supplementary table S1).

The crude prevalence of diseases by center and climatic zone is shown in figure 1. Allergic rhinitis was more frequent in the mediterranean zone with respect to the subcontinental zone, 27.6% vs 25.3% (p=0.02). Across centers, the highest prevalence was in Salerno (29.0%; 95%CI: 26.9-31.1%) and the lowest in Verona (24.4%; 95%CI: 22.5-26.5%). Similarly, asthma prevalence was higher in the mediterranean than in the subcontinental zone (11.3% vs 9.2%, p=0.001) and it ranged from 8.3% (95%CI: 6.7-10.2%) in Pavia to 12.5% (95%CI: 10.7-14.4%) in Sassari.

Pollen concentrations in 2007-2008 for each center are shown in supplementary figure S2. The distributions of pollen exposure indicators by center and climatic zone are reported in table 1 and supplementary table S2. With respect to the subcontinental zone, in the mediterranean zone the number of days with pollens above the low concentration threshold was higher for *Urticaceae*, *Oleaceae* and *Cupressaceae,* and lower for *Betula* and *Ambrosia* (Figure 2); the number of days at high concentration was higher for *Poaceae*, *Oleaceae* and *Cupressaceae,* and lower for *Betula* and *Ambrosia*.

The number of days with specific pollens above the low threshold were not significantly associated with the prevalence of allergic rhinitis (table 2); however, we found consistent negative associations (ORs below 1) of the number of days with high concentrations and pollen intensities with allergic rhinitis. In particular, these associations were statistically significant in the case of *Poaceae* (OR=0.977, p=0.024, for days with high concentrations; OR=0.983, p=0.025, for pollen intensity) and *Cupressaceae* (OR= 0.996, p=0.047 for pollen intensity) (table 2). When looking at exposure indicators based on concentrations of at least one or two pollens (figure 3), we found that centers with a longer period with high concentrations had significantly less allergic rhinitis: disease prevalence was 1.1% and 2.6% lower per 10 more days per year with high concentrations of at least one (OR=0.989, p=0.024) or at least two pollens (OR=0.974, p=0.028), respectively. The associations for the number of days with pollens above the low threshold were negative and borderline significant (figure 3).

The associations between pollen exposure indicators and asthma were not consistent (ORs were both greater and lower than 1), and none was statistically significant (table 2 and figure 3).

The sensitivity analyses performed using the indicators calculated over the specific years of questionnaire administration confirmed the main analyses (supplementary Figure S3), although there were some shifts in statistical significance in pollen-specific associations (supplementary tables S3 and S4).

DISCUSSION

In this cross-sectional multicenter study we report evidence on the ecologic associations between pollen exposure indicators and the prevalence of allergic respiratory diseases in young adults from the general Italian population. Our disease definitions were based on widely used questions in international epidemiological surveys such as ECRHS (Burney et al., 1994) or GA_2LEN (Björnsson et al., 1994). We were able to account for many individual determinants that are known risk factors for allergic respiratory disease and that may have confounded the associations.

Overall, our findings indicate that the centers with a higher pollen load (i.e. days with high concentrations and pollen intensities) had a lower prevalence of allergic rhinitis, and this result was fairly consistent across the different analyses carried out. The presence (i.e. concentrations above the low thresholds) of specific pollens was not consistently associated with the prevalence of allergic rhinitis, although borderline significant negative associations of the number of days with at least one/two pollens suggest a lack of statistical power.

The fact that the centers with a higher pollen load had a lower prevalence of allergic rhinitis may seem counterintuitive, because of the well-known adverse short-term effects of aeroallergens, which induce nasal and conjunctival symptoms in sensitized subjects, increase hospital and emergency room admissions for asthma, and even respiratory and cardiovascular mortality (Bono et al. 2016; Brunekreef et al., 2000; Caillaud et al., 2014; Cakmak et al., 2012; Cirera et al. 2012; Eriksson and Holmen, 1996; Sakaida et al., 2014; Tobias et al. 2004). However, data on the long-term effect of pollens on the prevalence of allergic diseases are scarce and inconsistent. Our findings on adults are in agreement with an ecologic analysis on children within the ISAAC study (Burr et al., 2003), where the prevalence of allergic rhinitis was inversely associated with pollen exposure. The authors hypothesized that childhood exposure to high pollen concentrations might give some protection against respiratory allergy. This mechanism has also been proposed to explain the induction of tolerance to perennial allergens in children (Ownby et al., 2003). On the other hand, another study performed in the US found a positive association between total pollen counts and hay fever in children (Silverberg et al., 2015). The lack of a positive relationship between pollens and rhinitis may also entail that other risk factors, such as exposure to perennial allergens, are important. About half of allergic rhinitis patients are sensitized to house dust mites, even if they only have a seasonal disease, and questionnaire definitions are unable to disentangle between seasonal and perennial rhinitis in the absence of clinical data (Olivieri et al., 2002).

To our knowledge, only one ecologic study in Japan assessed the long-term association between pollens and asthma (Yoshida et al., 2013). It documented a positive association between cedar pollen counts and childhood asthma prevalence. In our study on adults, there was no association between pollen concentrations and asthma prevalence. In a recent study on the same population, we reported that there is a substantial variability of asthma prevalence in Italy, and that this variability can be explained to a large extent by a "climate indicator" obtained by factor analysis on data from all 110 main Italian cities (Pesce et al., 2016). In Italy, asthma prevalence is greater in areas where annual temperatures are higher and temperature ranges are smaller, which are typical features of the mediterranean climate (de Marco, 2002; Zanolin et al. 2004; Pesce et al., 2016). The effect of climate may be partly related to the levels of perennial allergens, as indoor levels of house dust mite allergens are lower in areas with colder winters (Zock et al., 2006), but dietary and lifestyle determinants may also have a role (Pesce et al. 2016).

We observed a distribution of pollen concentrations that is consistent with the pattern of vegetation in the country. *Oleaceae* (e.g. olive, ash, jasmine) and *Cupressaceae* (e.g. cypress) are typical of the temperate and warm region, whereas *Betula* (birch) and *Ambrosia* (ragweed) are more common in northern Italy. *Poaceae* (e.g. grass) and

Urticaceae (e.g. nettle), the most common herbaceous families in Italy, are spread all over the peninsula, given their ability to grow in very different climatic conditions. *Coryloideae* (e.g. hornbeam, hophornbeam, and hazel) are mainly spread in the central and northern regions (Pre-Alps and Apennines). Among the indicators calculated, the number of days with pollens above the low thresholds represents the presence of measurable pollen levels during a year, under the hypothesis that even small pollen concentrations may affect disease prevalence at the population level. On the other hand, the number of days with high concentrations and pollen intensity are proxies of pollen load especially during the pollination season.

There was agreement between the analyses based on pollen data from 2007-08 and the analyses of data matched to the years of questionnaires administration, supporting that the results are robust to the method used to derive pollen exposure indicators. Also, the analyses based on pollen-specific concentrations and on concentrations of any pollen type were in a relative agreement. Differences in statistical significance may be explained by the low power due to the small number of centers included.

In the interpretation of our study findings, caution should be used. Diseases were defined using simple questionnaire items, since no clinical or laboratory data were available, and we had no information on the confirmation of allergic rhinitis by a doctor. Nonetheless, Olivieri et al. have documented that atopy is present among most (79%) of subjects reporting allergic rhinitis, and only in a minority (21%) of subjects who do not report allergic rhinitis (Olivieri et al., 2002). The ecologic associations observed at the center level may not reflect biologic associations at the individual level (the “ecologic fallacy”, Rothman et al., 2008). In particular, our study was unable to consider the spatial variability in pollen distribution within centers. As a consequence, we have no direct evidence to test the hypothesis that a more exposed individual is more likely to have the disease. As we only had data from a small number of centers, we had limited power to investigate center-level variables (such as pollen concentrations), we could only study one center-level variable at a time, and we had to assume that these variables had linear effects on log odds of disease. Since we had no data on air pollution exposure, we adjusted the analyses for self-reported exposure to traffic. Besides, we acknowledge that using data from Napoli Portici to derive pollen indicators for Salerno may have introduced further exposure misclassification (Katelaris et al., 2004).

In conclusion, evidence from this study does not support that allergic rhinitis and asthma are more frequent in centers with a greater pollen concentration. This suggests that, while pollen exposure is known to trigger acute effects in sensitized individuals, allergen sensitization and the development of allergic diseases may be less influenced by living in areas with a greater pollen concentration. Whether these ecologic associations hold at the individual level is still unknown.

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Daily data on pollen concentrations for the years described in figure S1 are available for research purposes upon approval of the GEIRD Steering Committee, in line with the data sharing principle of the GEIRD study (www.geird.org), and the previously mentioned institutions. For data queries please contact the corresponding author.

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Tables

Table 1. Average number of days per year with pollens above the relative low/high concentration threshold in 2007-2008 by study center.

Pollen		Centers					
Taxa	**Concentration threshold**	**Verona**	**Pavia**	**Torino**	**Ancona**	**Sassari**	**Salerno**
Poaceae	low	220	251	119	215	122	131
	high	31	47	2	60	75	1
Urticaceae	low	213	207	119	263	359	303
	high	46	73	3	23	64	17
Oleaceae	low	67	153	77	125	156	107
	high	9	9	14	20	66	9
Cupressaceae	low	74	81	50	122	233	85
	high	13	7	2	30	97	4
Coryloideae	low	105	116	79	107	82	92
	high	3	10	1	15	0	2
Betula	low	114	90	71	40	0	38
	high	16	17	1	7	0	0
Ambrosia	low	78	121	40	20	0	40
	high	0	25	1	0	0	0

Table 2. Associations† of the number of days per year with pollens above the low/high concentration threshold and pollen intensities (2007-2008) with allergic rhinitis.

Pollen Taxa	N. days above low threshold	N. days above high threshold	Pollen intensity
	OR (95% CI) per 10 days	OR (95% CI) per 10 days	OR (95% CI) per 1000 grains/m^3
Poaceae	0.994 (0.983-1.005)	**0.977 (0.958-0.997)**	**0.983 (0.969-0.998)**
Urticaceae	0.991 (0.978-1.004)	0.979 (0.958-1.001)	0.991 (0.983-1.000)
Oleaceae	0.986 (0.968-1.005)	0.977 (0.949-1.006)	0.996 (0.992-1.001)
Cupressaceae	0.989 (0.978-1.000)	0.983 (0.965-1.001)	**0.996 (0.991-0.999)**
Coryloideae	0.985 (0.944-1.027)	0.973 (0.881-1.074)	0.977 (0.919-1.038)
Betula	1.006 (0.978-1.036)	0.940 (0.851-1.038)	0.969 (0.921-1.021)
Ambrosia	1.000 (0.979-1.022)	0.977 (0.909-1.051)	0.970 (0.881-1.067)

† OR were adjusted for gender, age, smoking habits, education level, heavy traffic near home, season of response, type of contact, percentile rank of response, climatic zone. OR with p<0.05 are in bold

Table 3. Associations† of the number of days per year with pollens above the low/high concentration threshold and pollen intensities (2007-2008) with asthma.

Pollen Taxa	N. days above low threshold	N. days above high threshold	Pollen intensity
	OR (95% CI) per 10 days	OR (95% CI) per 10 days	OR (95% CI) per 1000 grains/m^3
Poaceae	0.995 (0.979-1.012)	1.011 (0.981-1.042)	1.010 (0.989-1.033)
Urticaceae	1.014 (0.994-1.035)	1.016 (0.983-1.051)	1.008 (0.994-1.022)
Oleaceae	0.999 (0.971-1.029)	1.030 (0.987-1.074)	1.005 (0.998-1.012)
Cupressaceae	1.012 (0.995-1.028)	1.019 (0.993-1.047)	1.004 (0.998-1.011)
Coryloideae	0.975 (0.915-1.041)	0.907 (0.781-1.054)	0.943 (0.859-1.036)
Betula	0.988 (0.946-1.032)	0.992 (0.850-1.157)	0.998 (0.920-1.082)
Ambrosia	0.987 (0.954-1.021)	0.954 (0.849-1.071)	0.937 (0.803-1.093)

† OR were adjusted for gender, age, smoking habits, education level, heavy traffic near home, allergic rhinitis, season of response, type of contact, percentile rank of response, climatic zone.

Figures

Figure 1. Crude prevalence of allergic rhinitis (A) and asthma (B) by study center and climatic zone.

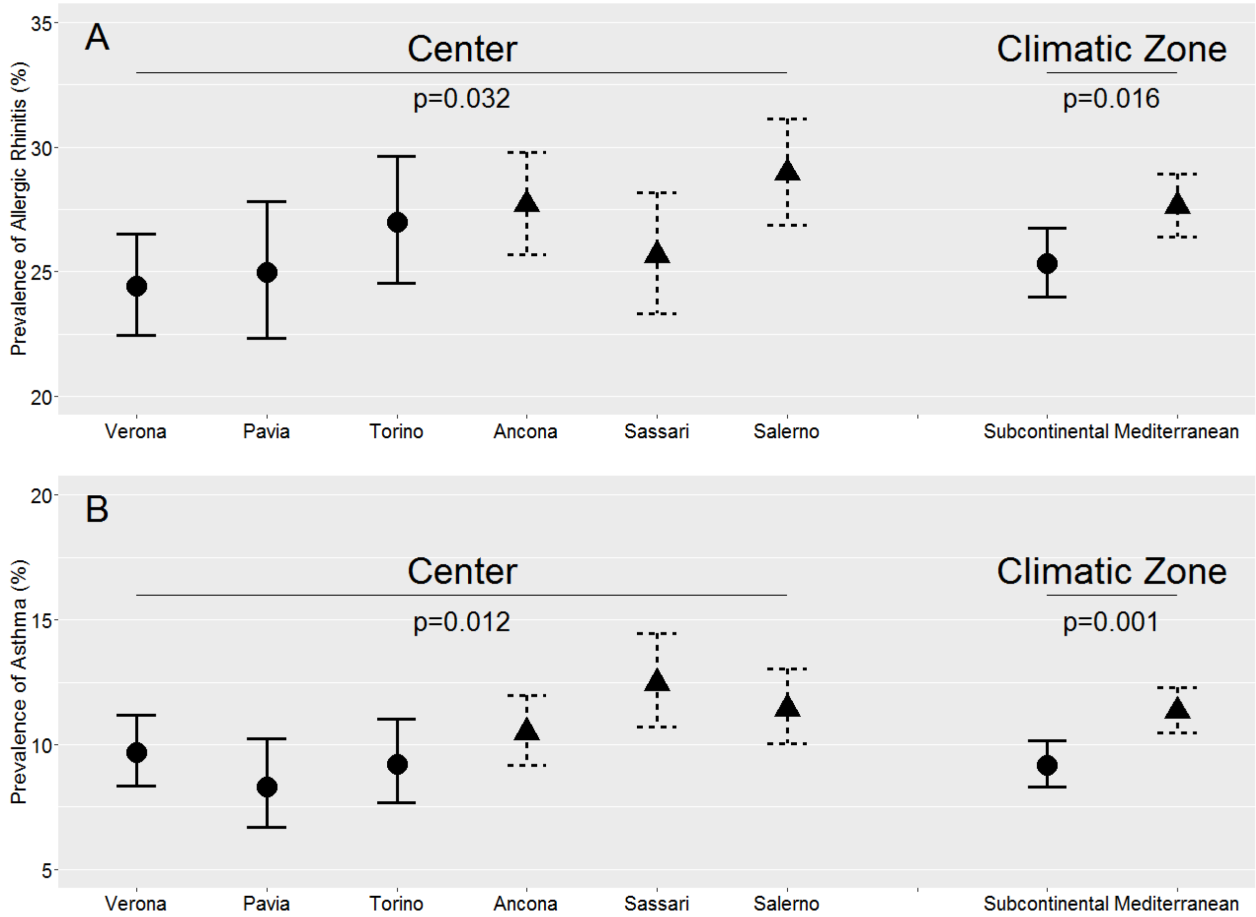


Figure 2. Average number of days per year with pollen above the low and high thresholds in 2007-2008 by climatic zone.

Figure 3. Associations[†] between the number of days per year with at least one/two pollens (2007-2008) above the low/high thresholds and the prevalence of allergic rhinitis and asthma.

[†] OR for 10 days/year. Adjusted for gender, age, smoking habits, education level, heavy traffic near home, season of response, type of contact, percentile rank of response, climatic zone. OR for asthma are also adjusted for allergic rhinitis.

ONLINE SUPPLEMENTARY MATERIAL

Table S1. Distribution of subjects' characteristics by study center and climatic zone.

		Centers							Climatic zone		
Covariates		Verona (n=1746)	Pavia (n=966)	Torino (n=1205)	Ancona (n=1866)	Sassari (n=1245)	Salerno (n=1806)	p*	Subcontinental (n=3917)	Mediterranean (n=4917)	p*
Male gender(%)		47.0	46.4	44.4	48.4	47.7	49.8	0.082	46.0	48.8	0.011
Age (years) (median; IQR**)**		35.4; 11.5	35.9; 10.6	36.7; 12.1	34.3; 11.7	35.0; 11.2	34.4; 12.3	<0.001	35.9; 11.5	34.5; 11.8	<0.001
Smoking habits (%)	non smokers	54.4	58.3	58.4	58.4	52.7	60.2	<0.001	56.6	57.6	0.002
	ex-smokers	19.1	17.8	15.5	15.7	19.6	11.1		17.7	15.0	
	Smokers (<15 packs-year)	16.0	14.1	15.1	15.0	14.8	15.4		15.2	15.1	
	smokers (≥15 packs-year)	10.6	9.9	11.1	10.9	13.0	13.3		10.5	12.3	
Level of education(%)	lower	21.8	23.7	19.2	17.3	25.2	13.6	<0.001	21.5	20.0	<0.001
	higher	52.4	51.5	51.3	59.4	48.2	52.8		51.9	54.2	
	degree	25.8	24.8	29.5	23.3	26.5	33.6		26.7	27.9	
Frequent/ constant heavy traffic near home(%)		39.7	28.4	53.9	41.2	37.6	53.7	<0.001	41.2	44.8	0.001
Season of response(%)	spring	33.9	57.7	45.6	59.8	55.2	21.9	<0.001	43.4	44.7	<0.001
	summer	3.6	23.3	32.1	24.5	8.7	16.9		17.2	17.7	
	autumn	50.3	17.9	18.3	1.3	30.4	34.3		32.5	20.8	
	winter	12.1	1.1	4.0	14.4	5.8	26.9		6.9	16.8	
Type of contact (%)	postal (vs. phone)	84.1	93.3	93.7	100.0	85.5	72.0	<0.001	89.3	86.0	<0.001

IQR, interquartile range. *p-values were computed using Pearson's Chi-square test, except for variable age where Kruskal-Wallis test was used.

Table S2. Average pollen intensities in 2007-2008 (grains/m^3) by study center and climatic zone.

	Centers						Climatic zone	
Pollen taxa	**Verona**	**Pavia**	**Torino**	**Ancona**	**Sassari**	**Salerno**	**Subcontinental**	**Mediterranean**
Poaceae	3642	5805	731	7341	11754	590	3393	6561
Urticaceae	13361	17658	1581	7147	18245	6652	10867	10681
Oleaceae	865	1098	895	2190	34973	1124	952	12762
Cupressaceae	4988	3456	1104	20626	42302	2272	3183	21733
Coryloideae	826	2440	555	2892	753	794	1273	1479
Betula	3241	3824	424	791	0	110	2496	300
Ambrosia	135	2003	136	62	0	21	758	27

Table S3. Associations† of the number of days per year with pollens above the low/high thresholds and pollen intensities (for the years of questionnaire administration in each center) and allergic rhinitis.

Pollen Taxa	**N. days above low threshold**	**N. days above high threshold**	**Pollen intensity**
	OR (95% CI) per 10 days	OR (95% CI) per 10 days	OR (95% CI) per 1000 grains/m^3
Poaceae	0.998 (0.987-1.009)	0.980 (0.956-1.004)	**0.980 (0.964-0.998)**
Urticaceae	0.993 (0.982-1.004)	**0.979 (0.959-0.999)**	**0.989 (0.979-0.999)**
Oleaceae	0.985 (0.964-1.006)	0.967 (0.935-1.000)	0.997 (0.994-1.001)
Cupressaceae	0.990 (0.980-1.000)	0.986 (0.971-1.002)	**0.995 (0.990-0.999)**
Coryloideae	0.983 (0.951-1.016)	0.968 (0.869-1.077)	0.964 (0.894-1.039)
Betula	0.996 (0.976-1.016)	0.938 (0.842-1.044)	0.971 (0.919-1.026)
Ambrosia	1.004 (0.981-1.027)	0.979 (0.920-1.042)	0.972 (0.894-1.056)

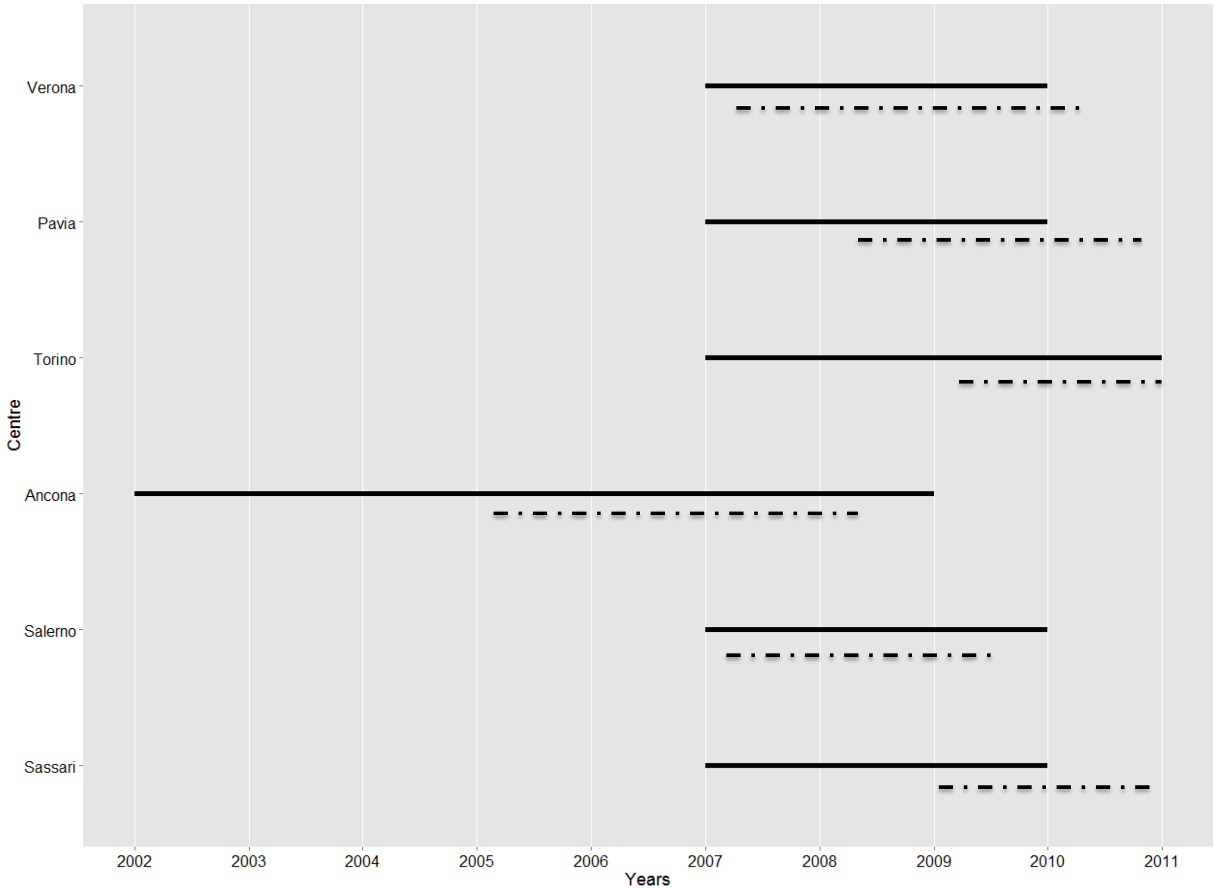
† OR were adjusted for gender, age, smoking habits, education level, heavy traffic near home, season of response, type of contact, percentile rank of response, climatic zone. OR with $p<0.05$ are in bold

Table S4. Associations† of the number of days per year with pollens above the low/high thresholds and pollen intensities (for the years of questionnaire administration in each center) and asthma.

Pollen Taxa	**N. days above low concentration threshold**	**N. days above high concentration threshold**	**Pollen intensity**
	OR (95% CI) per 10 days	OR (95% CI) per 10 days	OR (95% CI) per 1000 grains/m^3
Poaceae	0.993 (0.977-1.010)	1.010 (0.974-1.048)	1.014 (0.989-1.040)
Urticaceae	1.013 (0.996-1.030)	1.006 (0.975-1.038)	1.006 (0.990-1.022)
Oleaceae	0.999 (0.966-1.032)	1.031 (0.981-1.082)	1.004 (0.999-1.008)
Cupressaceae	1.011 (0.996-1.026)	1.017 (0.994-1.040)	1.004 (0.996-1.011)
Coryloideae	1.006 (0.954-1.060)	0.903 (0.768-1.063)	0.944 (0.842-1.059)
Betula	1.001 (0.971-1.033)	1.032 (0.872-1.222)	1.022 (0.937-1.115)
Ambrosia	0.982 (0.948-1.017)	0.954 (0.863-1.055)	0.934 (0.817-1.068)

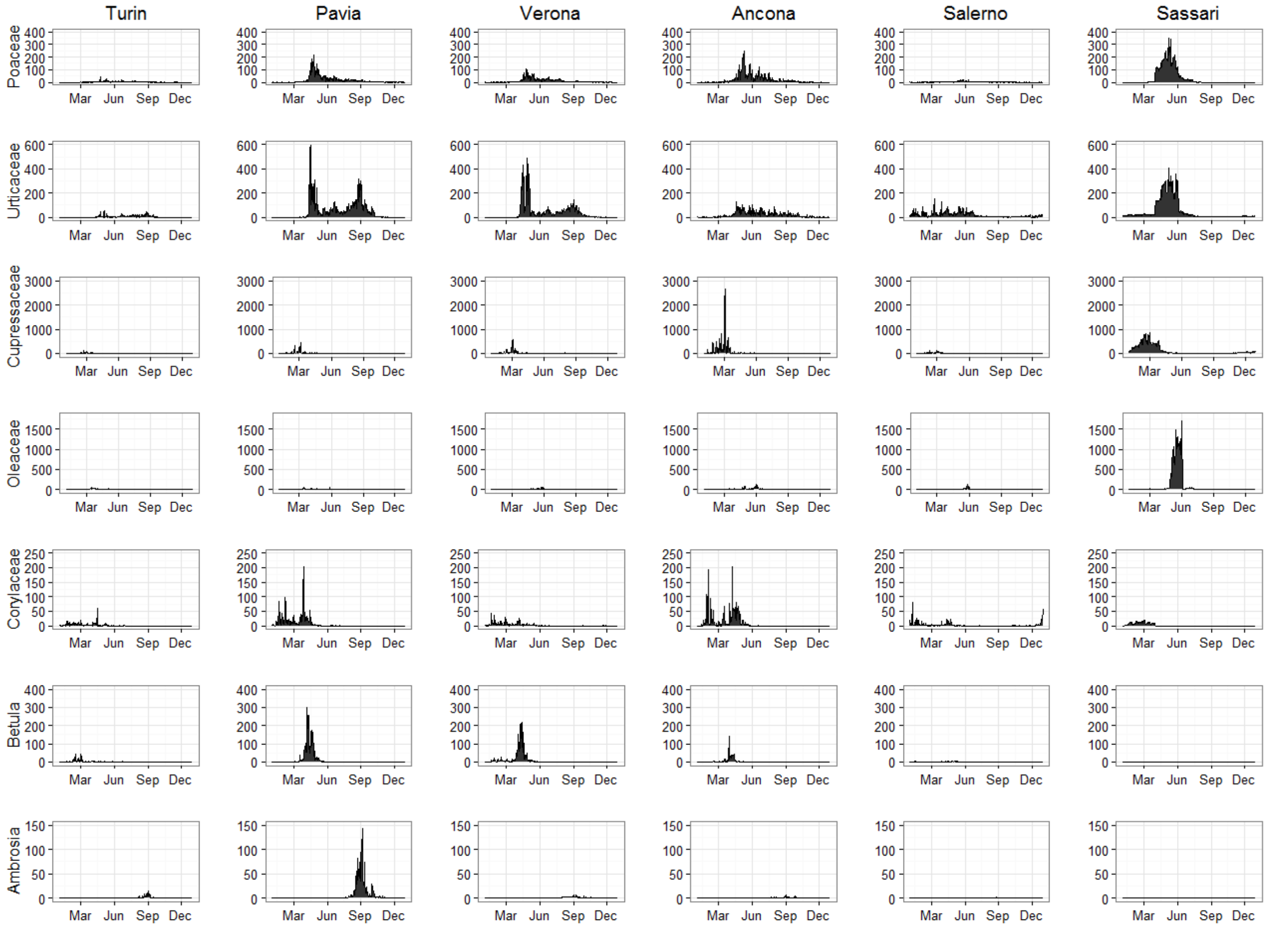
† OR were adjusted for gender, age, smoking habits, education level, heavy traffic near home, allergic rhinitis, season of response, type of contact, percentile rank of response, climatic zone.

Figure S1. Period when questionnaire and pollen data were collected by study center. †



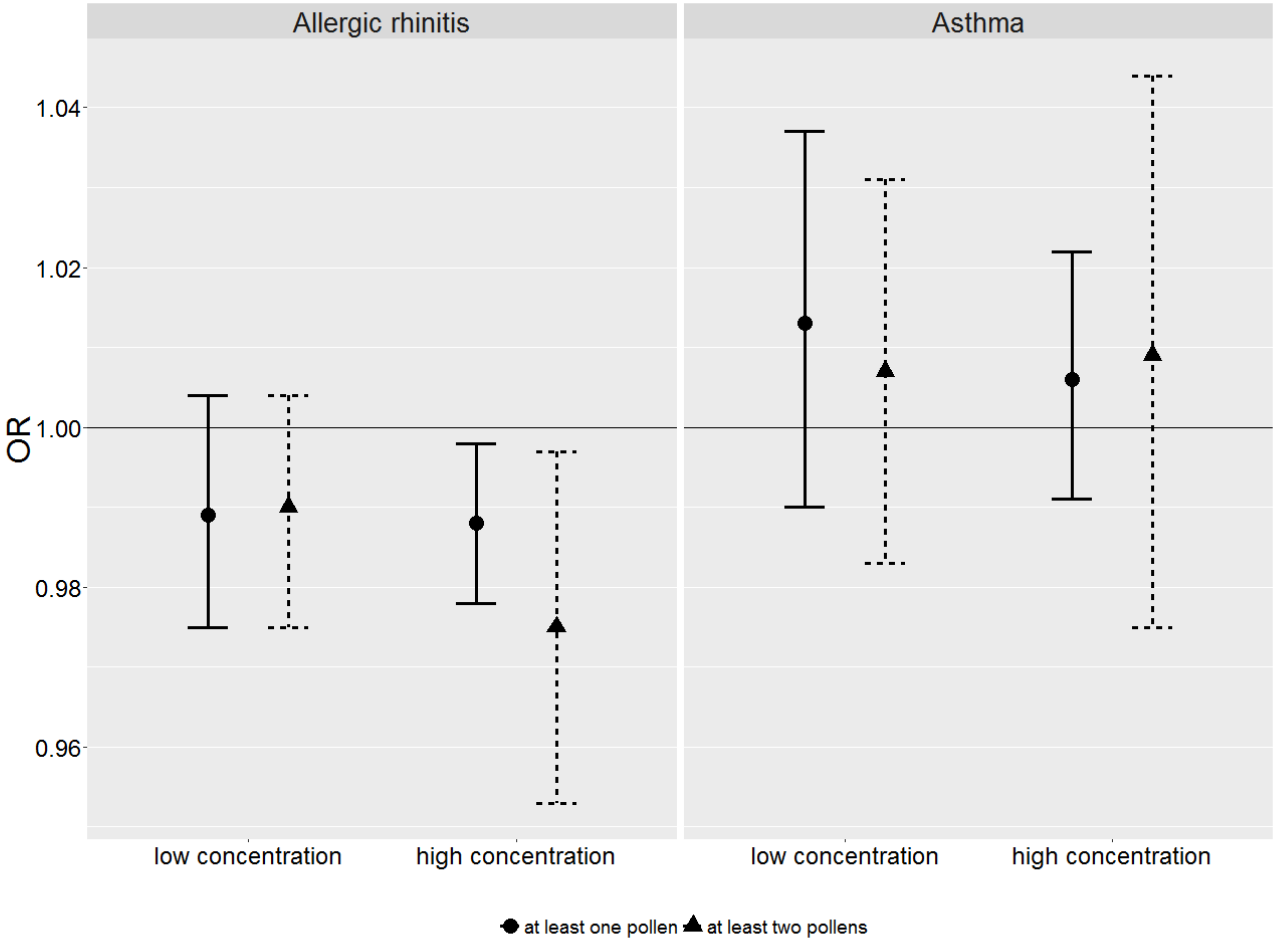
† Solid lines represent pollen data, dashed-dot lines represent the periods when questionnaires were collected.

Figure S2. Distribution of daily† pollen concentrations in 2007-2008 (grains/m^3) by study center.



† pollen concentrations for a given calendar day were averaged between 2007 and 2008 for descriptive purposes

Figure S3. Associations† of the number of days with at least one/two pollens above the low/high thresholds (for the years of questionnaire administration in each center) with the prevalence of allergic rhinitis and asthma.



† OR for 10 days/year. Adjusted for gender, age, smoking habits, education level, heavy traffic near home, season of response, type of contact, percentile rank of response, climatic zone. OR for asthma are also adjusted for allergic rhinitis.