

Aeroallergens: a comparative study of two monitoring methods

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Abstract Olive and grass pollen grains are the major causes of hay fever in the Mediterranean region. A number of samplers and methods have been developed in recent years in order to obtain reliable data regarding airborne allergen concentrations. This paper reports on a detailed comparison of two samplers—Cyclone and ChemVol—and on the parameters that could influence their efficiency. Airborne concentrations of two key olive and grass allergens, Ole e 1 and Phl p 5, respectively, were monitored over two years with different weather patterns, 2012 and 2014. Allergenic particles were quantified by ELISA assay, and results were compared with pollen concentrations monitored using a Hirst-type volumetric spore trap over the same study periods. The influence of weather-related parameters on local airborne pollen and allergen concentrations was also analysed. Although

a positive correlation was detected between results obtained using the two samplers during the pollen season, results for the cumulative annual Allergen Index varied considerably. The two samplers revealed a positive correlation between pollen concentrations and both minimum temperature during the warmer year (2012) and maximum temperature during the cooler year (2014); a negative significant correlation was observed in both cases with rainfall and relative humidity. In summary, although some differences were observed between the two samplers studied, both may be regarded as suitable for allergen detection.

Keywords Olive · Grass · Aeroallergens · Cyclone sampler · ChemVol sampler · Airborne pollen

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1 Introduction

Pollen allergy is among the most prevalent causes of seasonal respiratory disease in Europe. Exposure to allergens derived from pollen grains, microsome particles or other parts of the plant, is an important risk factor for the development of allergic symptoms such as rhinitis and conjunctivitis (Fernández-Caldas et al. 1992; D'Amato et al. 1991) and of asthma (Eder et al. 2006; Cecchi et al. 2010); it is also associated with allergen sensitization (De Weed et al. 2002; Brito et al. 2011). Air sampling has traditionally been used to analyse atmospheric bioaerosol composition

(Mandrioli et al. 1998), while data on airborne pollen concentrations have helped the medical community to determine the effects of allergen exposure on patient symptoms (Beggs 1998; D'Amato 2001) and to draw up pollen calendars for different geographical areas (D'Amato et al. 2007; Martínez-Bracero et al. 2015). However, recent research using immunochemical methods to quantify pollen allergens has shown that airborne pollen concentrations are not always an accurate indicator of allergen exposure (Buters et al. 2010; De Linares et al. 2010; Fernández-González et al. 2011; Galán et al. 2013; Plaza et al. 2016a), largely because aeroallergen concentrations are determined not only by pollen emission but also by various environmental and meteorological parameters influencing exposure during pollen flight and affecting the release of allergens from pollen (e.g. Fernández-González et al. 2011; Cecchi 2013; Galán et al. 2013; Plaza et al. 2016b). Accordingly, aerobiologists in a number of countries are now using immunological techniques to study aeroallergens, although classical samplers remain in operation.

A wide variety of sampling devices are currently in use (Mandrioli et al. 1998; Levetin 2004; Scheifinger et al. 2013), and new methods and instruments are continually being developed. It is likely that no single method is appropriate for all bioaerosols or for all applications. Recent research into aeroallergens has made use of several different samplers, including the Cyclone (Emberlin 1995), the Andersen (1958) and the ChemVol (Demokritou et al. 2002). Moreover, standard protocols are not yet available for some of these techniques. For a full understanding of the mechanisms involved in disease development, allergen exposure needs to be carefully estimated; for this purpose, accurate and precise sampling is essential.

The instruments most widely used for airborne biological particle sampling are impaction samplers, which separate particles from the air stream by using the inertia of the particles themselves, resulting in their deposition and retention on surfaces smeared with sticky substances, or others with non sticky substrates like aluminium or filters. The impaction sampler most commonly utilized in Europe for pollen and spore capture is the 7-day Hirst-type volumetric spore trap (Hirst 1952). A variant known as the sieve impactor is widely used for allergen collection; examples include the Andersen (e.g. De Linares et al. 2007, 2010) and ChemVol (e.g. Buters et al. 2010, 2012, 2015; Galán

et al. 2013; Jochner et al. 2015) samplers. Other collection methods include Cyclone sampling (e.g. Rodríguez-Rajo et al. 2011; Alcázar et al. 2015; Plaza et al. 2016a, b), the less-common electrostatic precipitation technique (Mandrioli et al. 1998) and a static size-selective bioaerosol sampler (SSBAS) (Rantio-Lehtimäki et al. 1994).

Allergen monitoring provides valuable data for allergy research, but can prove both expensive and time-consuming; the use of one type of sampler or another may make a significant difference in this respect, provided that both yield valid results. Accurate aeroallergen detection requires the adoption of a standard method for capturing airborne particles that enables subsequent application of appropriate techniques.

At present, the most widespread pollen allergies are those induced by the grass family (Poaceae), which comprises more than 600 genera and over 10,000 species. In Córdoba (southern Spain), only a small number of Poaceae species contribute significantly to overall airborne pollen concentrations: *Arrhenatherum album*, *Dactylis glomerata*, *Lolium rigidum*, *Trisetaria panicea* and *Vulpia geniculata* (León-Ruiz et al. 2011; Cebrino et al. 2016). Olive pollen, produced by a single tree species, is also among the most common causes of pollinosis in the Mediterranean Region and in particular in Córdoba (Sánchez-Mesa et al. 2005b; Cebrino et al. 2017).

This paper reports on a comparison of two samplers used to detect the major grass and olive allergens, Phl p 5 and Ole e 1. The specific aims of the study were: to compare the results obtained using the two samplers with airborne pollen monitored using a Hirst-type volumetric spore trap and to examine the influence of weather-related parameters on airborne pollen and aeroallergen concentrations.

2 Materials and methods

2.1 Study area

The city of Córdoba is located in south-western Spain (37°53'N, 4°45'O), 123 m a.s.l. It has a Mediterranean climate with some continental features. The annual mean temperature is 18.1 °C, and the annual average rainfall is 591 mm (30-year averages, 1982–2011, data from the Spanish Meteorology Agency, AEMET—<http://www.aemet.es>).

2.2 Airborne pollen and aeroallergen monitoring

The study was carried out during the spring of 2012 and 2014, two years with different meteorological characteristics: 2012 was drier and warmer than 2014.

Three samplers were used: one for airborne pollen and two for airborne aeroallergen capture. All three were located close to each other, at 22 m above ground level in Rabanales University Campus, in the northeast of the city of Córdoba.

Airborne pollen sampling was performed using a Hirst-type volumetric spore trap (Hirst 1952), in accordance with the Spanish Aerobiology Network (REA) Management and Quality Manual (Galán et al. 2007) and the minimum requirements proposed by the European Aerobiology Society (EAS) (Galán et al. 2014). Data were expressed as daily average number of pollen grains per cubic metre of air. The pollen season was defined following Velasco-Jiménez et al. (2013) for olive and Plaza et al. (2016b) for grasses: the start-date was defined as the first day with over 50 pollen grains/m³ in olive and 10 pollen grains/m³ for grasses, followed by the same concentrations for at least five consecutive rain-free days; the end-date was defined as the last day with this daily value, followed by five consecutive days with lower values.

The major allergens Ole e 1 and Phl p 5 were detected using two different samplers: the wind-oriented low-volume Burkard multi-vial Cyclone sampler and the high-volume ChemVol cascade impactor. The two samplers use different methods:

1. Low-volume Burkard multi-vial Cyclone sampler. Particles were collected in a 1.5-ml Eppendorf vial located in a carousel, through a gentle vortex flow of air at 16.5 L/min (Fernández-González et al. 2010; Rodríguez-Rajo et al. 2011). Samples corresponding to daily data were stored at -40 °C until extraction.
2. ChemVol 2400 high-volume cascade impactor (Albany, NY, USA). Flow was adjusted to 800 L/min with a Rupprecht and Patashnick flow measuring system (Albany) according to manufacturer's instructions. Prewashed polyurethane foam served as impacting substrate. Particulate matter (PM) in ambient air is fractionated into PM < 10 µm and PM < 2.5 µm. However, for this study, the sum of allergens in both fraction stages was used. Each stage of a ChemVol

cascade impactor featured three identical, clearly separated collection slits. Thus, the impacting substrates were cut into three identical parts and stored separately in polypropylene tubes at -80 °C until extraction (Buters et al. 2010).

Daily samples were obtained with both samplers; however, with the ChemVol sampler, daily data represented the period from 01:00 to 24:00 and with the Cyclone sampler, the period from 12:00 a.m. to 11 a.m. of the following day. Daily pollen concentrations therefore had to be adapted for purposes of comparison.

The study was carried out during the olive and grass pollen seasons plus two weeks before and after the season, in order to detect possible aeroallergens outside the flowering period.

2.3 Extraction and quantification of airborne allergens

Aeroallergen concentrations in aerosol samples recorded by both samplers were quantified using ELISA Double Technical phase, better known as ELISA double sandwich, since the antigen extract obtained is trapped between two antibodies. Monoclonal (5A3 for olive and 10B1 and 10C12 for grasses) and polyclonal antibodies were used as capture and detection antibodies, respectively, in each analysis. The same protocol was used for detecting Ole e 1 and Phl p 5 in both samplers. However, there were some differences in the sample extraction method.

1. Extraction of samples from the Cyclone sampler was performed following Takahashi et al. (2001) as modified by Moreno-Grau et al. (2006) and Plaza et al. (2016a, b): samples collected in 1.5-ml Eppendorf vials were centrifuged for 3 min at 6000 rpm, so that the particles were deposited on the bottom. Samples were then resuspended in 120 µl of extraction buffer (50 mmol/L phosphate buffer at pH 7.4, containing 150 mmol/L sodium chloride, 125 mmol/L ammonium bicarbonate, 3 mmol/L EDTA and 0.005% Tween 20) for 2 h at room temperature under stirring, and centrifuged at 4000 rpm for 10 min. The supernatant was then collected, taking care not to take the pellet, and placed in a new Eppendorf. Four 30 µl aliquots were obtained, in order to avoid having to

thaw the sample several times if more than one analysis was to be performed; aliquots were stored at -40°C until analysis.

2. Extraction of samples from the ChemVol sampler was performed following Buters et al. (2010). Polyurethane impacting substrates from two different-sized filters (XL for Particulate Matter (PM) greater than $10\text{ }\mu\text{m}$ —PM $< 10\text{ }\mu\text{m}$ —and M filter for PM greater than $2.5\text{ }\mu\text{m}$ but less than $10\text{ }\mu\text{m}$ in diameter— $10 > \text{PM} > 2.5$, as described by Demokritou et al. (2002)) were extracted for 4 h in $0.1\text{ M NH}_4\text{HCO}_3$ pH 8.1 with 0.1% bovine serum albumin (BSA) in a head-overhead rotator. Later, extracts were aliquoted, frozen, lyophilized and stored at -20°C until analysis. Before performing the ELISA, they were redissolved in 1/10 of the original volume in phosphate-buffered saline (PBS) pH 7.4 and diluted according to the amount of pollen obtained with the Hirst sensor. For each day, two filter segments (XL and M) were independently analysed; reported values were the sum of the two filter segments.

The ELISA double sandwich method used here was that previously described by Arilla et al. (2002, 2005, 2006), Moreno-Grau et al. (2006) and Rodríguez-Rajo et al. (2011). Briefly, 96-well microplates (Greinerbio-one, Germany) were coated with $100\text{ }\mu\text{l}$ of the corresponding monoclonal antibody (Bial Industrial Pharmaceutical, Spain) 5A3 for olive and 10B1 and 10C12 for grasses, 5 mg/ml diluted in PBS and incubated overnight at 4°C in a humid chamber. The next day, the plate was blocked with $200\text{ }\mu\text{l/well}$ of BSA-PBS-T (PBS + 1% BSA and 0.05% Tween 20) and incubated for 1 h at 37°C . Later, $100\text{ }\mu\text{l/well}$ of the calibration curve and sample extracts was diluted with BSA-PBS-T according to daily pollen concentration. Each assay was calibrated against purified natural Ole e 1 and Phl p 5 (generated with a series of dilutions in duplicate from 0.10 ng/ml to 50 ng/ml) and included a positive control. All extracts were diluted such that the values were within the linear range of the calibration curve. Moreover, all samples were determined at least in duplicate. After washing the plate with $200\text{ }\mu\text{l}$ of PBS-T buffer (PBS + Tween 20 0.05%), $100\text{ }\mu\text{l/well}$ of polyclonal antibody (Biotinylated rabbit anti Ole e 1 and Biotinylated rabbit anti Phl p 5, Bial Industrial

Farmaceutica, Spain, 0.5 mg/ml) and peroxidase conjugated streptavidin (0.25 mg/ml in PBS-BT) were added sequentially; plates were incubated for 1 h at 37°C . Finally, wells were incubated for 10 min at room temperature with $100\text{ }\mu\text{l}$ TMB in the dark. The reaction was stopped by adding $100\text{ }\mu\text{l}$ of $3\text{ M H}_2\text{SO}_4$ and absorbance was measured at 450 nm .

In each case, the final curve was obtained as an average of the two absorbance readings, in order to minimize potential error. Samples were analysed in duplicate and daily concentrations were interpolated from the standard curve and expressed in picograms per cubic metre of air.

2.4 Meteorological data

Meteorological data for the study years were supplied by the Andalusian Regional Government Agroclimatic Information Network at the weather station on the outskirts of Córdoba city (UTM coordinates $37^{\circ}51'25''\text{ N}$, $04^{\circ}48'10''\text{ W}$).

2.5 Statistical analysis

Spearman's (nonparametric) correlation test was applied to identify potential correlations between Ole e 1 and Phl p 5 and the relevant pollen taxa and also to test for correlations between pollen concentrations and weather variables (temperatures, relative humidity, rainfall and wind speed), over the entire study period, including the pollen season and the two weeks before and after the pollen season; calculations were performed using the SPSS version 14 software package for Windows.

3 Results

Different weather patterns were recorded in the two study years. Before the pollen season (i.e. from January to March), 2012 was drier and warmer than 2014; during the pollen season, 2012 was considerably warmer than 2014 (Table 1).

Inter-year differences were observed with regard to pollen season timing and intensity. For both olive and grass, the pollen season was longer and the Pollen Index (PI) higher in 2014, while the peak day was also recorded earlier and the peak value was higher (Table 2).

Table 1 Yearly mean temperature, relative humidity, and total rainfall in Córdoba city (2012–2014)

	2012	2014
Before PS		
Mean temperature (°C)	10.3	9.4
Precipitation(mm)	33.2	225.6
Mean relative humidity (%)	64.6	84.3
During PS		
Mean temperature (°C)	21.9	19.4
Precipitation(mm)	111.9	93.8
Mean relative humidity (%)	55.6	62.6
PS pollen season		

Table 2 Characteristics of the 2012 and 2014 pollen seasons

	<i>Olea</i>		Poaceae	
	2012	2014	2012	2014
PS start	6–5	16–4	25–4	17–3
PS end	13–6	1–6	19–6	30–6
PS length (days)	39	47	56	106
PV	3654	4280	202	1180
PD	16–5	5–5	29–5	12–5
PI	20,625	40,060	3005	9296

PS pollen season, PI pollen index, PV peak value, PD peak day

Aeroallergens collected with the Cyclone and ChemVol samplers in 2012 and 2014 are shown in Fig. 1; allergen season characteristics are indicated in Table 3.

Comparable Ole e 1 and Phl p 5 allergen concentrations were recorded using both samplers during the pollen season. Although allergen season length was similar with both devices, the Cyclone sampler was more sensitive to low pollen concentrations before and after the pollen season. Aeroallergens concentrations recorded with the Cyclone were generally higher than those recorded with the ChemVol sampler. In 2012, the Cyclone sampler recorded 72.78% of the total Phl p 5 allergen detected with both samplers, and 65.98% of the total Ole e 1. In 2014, differences were less marked: the Cyclone sampler detected 56.32% for Phl p 5 and 51.51% for Ole e 1. It should also be noted that allergen peak values (PV) were higher with the Cyclone than with the ChemVol, except for Ole e 1 in 2014 (Table 3), when the Allergen Index (AI) was similar. Allergy Potency (AP) varied both between samplers and between study years, values obtained

with the Cyclone sampler being higher except for *Olea* in 2014, when similar values were obtained with the two devices. Results indicated high Ole e 1 allergen concentrations in 2012 with both samplers; values using the Cyclone sampler were twice as high in 2014 as in 2012 (Table 3). Higher values were recorded for AP (both Ole e 1 and Phl p 5) in 2012, though airborne pollen concentrations were lower; conversely, lower AP values were observed in 2014 when airborne pollen concentrations were higher.

Comparative curves plotting olive and grass pollen concentrations against their major allergens detected with both samplers in both study years (Figs. 2 and 3) displayed a similar oscillation; aeroallergen peaks were recorded on the same day as airborne pollen peaks, or within one or two days. On days with lower pollen concentrations, the Cyclone sampler was generally more sensitive; higher allergen concentrations were recorded for this sampler both before and after the pollen season.

Results revealed a significant positive correlation between aeroallergen concentrations and airborne pollen concentrations using both samplers (Table 4). However, this correlation displayed greater statistical significance with the ChemVol sampler, except in 2012 with olive, when the statistical significance was negligible.

In 2012, a positive significant correlation was observed between both allergens detected with the two samplers and minimum and mean temperature (Table 2); in 2014, however, significant correlation was observed only with mean and maximum temperature using the ChemVol, and with maximum temperature using the Cyclone, for Ole e 1 alone. For both allergens, a negative significant correlation was observed between allergen concentrations and both mean relative humidity and rainfall, in both study-years with the ChemVol but only in 2014 with the Cyclone. A positive significant correlation between allergen levels and wind speed was recorded in 2012 with both samplers, and a negative significant correlation in 2014.

4 Discussion

This study focused on the two major aeroallergens in South Europe, Ole e 1 and Phl p 5. Various samplers are currently used for aeroallergen monitoring. Here,

Fig. 1 Data for allergens (Ole e 1 and Phl p 5) collected by the Cyclone (CY) and ChemVol (CH) samplers

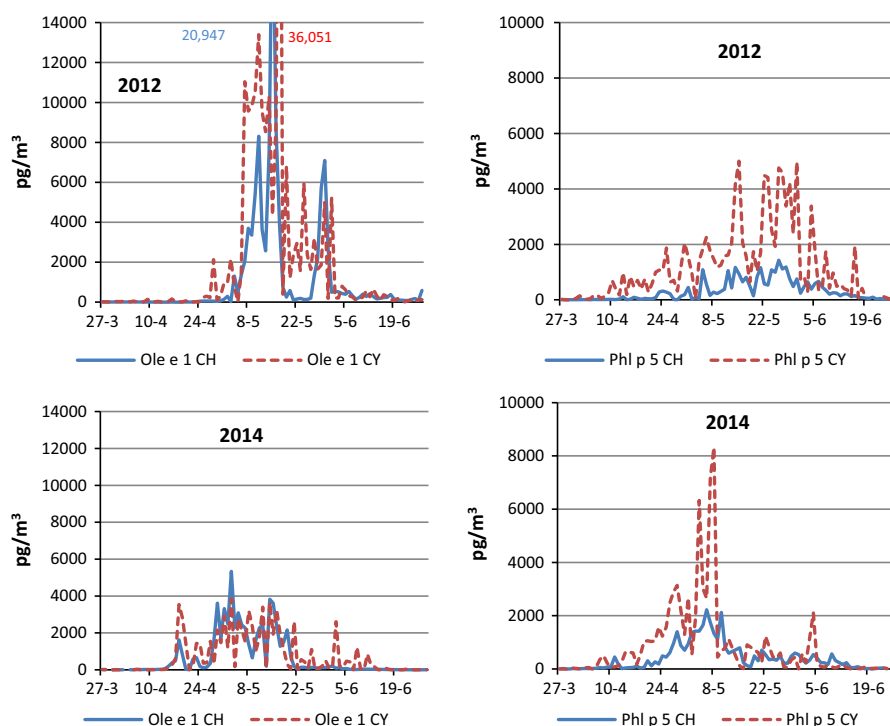


Table 3 Characteristics of the 2012 and 2014 allergen seasons

	Ole e 1				Phl p 5			
	2012		2014		2012		2014	
	ChemVol	Cyclone	ChemVol	Cyclone	ChemVol	Cyclone	ChemVol	Cyclone
PV	20,947.44	36,051.65	5334.59	3838.42	1428.08	5002.87	2222.48	8317.33
PD	15 May	17 May	3 May	3 May	26 May	15 May	10 May	12 May
AI	97,937.95	180,209.38	59,104.09	62,366.01	26,595.41	96,736.87	35,568.97	79,613.98
AP	4.75	8.74	1.48	1.56	8.85	32.19	3.83	8.56

PV peak value (pg/m³), PD peak day (date), AI allergen index (pg), AP allergy potency (pg/pollen)

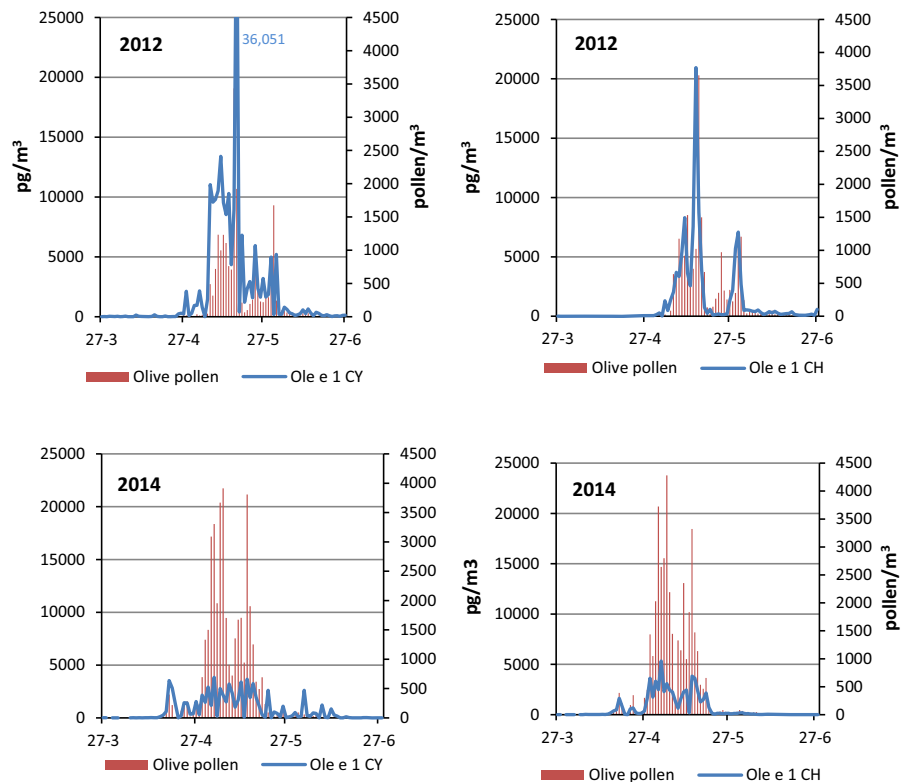
two samplers were compared: a low-volume Cyclone instrument and a high-volume ChemVol cascade impactor.

A number of authors have reported on allergen detection using the Cyclone sampler (e.g. Rodríguez-Rajo et al. 2011; Alcázar et al. 2015; Plaza et al. 2016a, b) and the ChemVol sampler (e.g. Buters et al. 2010, 2012, 2015; Galán et al. 2013; Jochner et al. 2015). However, no published papers assess the efficiency of the two samplers by comparison with airborne pollen concentrations different environmental conditions. One advantage of the Cyclone sampler

is its high efficiency over a wide range of captured particles, 90% for particles in the range of 1 micron (Emberlin 1995); the ChemVol sampler, for its part, enables particle size discrimination (Demokritou et al. 2002).

Two methodological differences are worthy of note. The Cyclone sampler has the advantage that Eppendorfs for allergen collection can be changed on a weekly basis, whereas the polyurethane foam served as impacting substrate with the ChemVol sampler must be changed daily during the pollen season. With the ChemVol buffer, ammonium bicarbonate, we can

Fig. 2 Ole e 1 allergen versus olive pollen collected by Cyclone (CY) and ChemVol (CH) samplers in 2012 and 2014



performed sample lyophilization during the extraction procedure, which can help to remove interfering substances disturbing the ELISA test as well as to increase sensitivity (Buters et al. 2008).

These differences apart results showed a clear correlation between aeroallergen concentrations detected with the two samplers (99%, $p < 0.01$), and an equally clear correlation between both devices and pollen concentrations (99%, $p < 0.01$). Higher allergen concentrations were detected with the Cyclone sampler, perhaps due to the stress undergone by pollen under vortex air flow into the Eppendorfs, which maybe triggers greater allergen release than in ambient air; however, this possible mechanical effect is not clear because the curve distribution was similar in both samplers not showing higher allergen concentration even when there was high pollen concentration. Another possible methodological explanation is that the extraction buffer used in the Cyclone protocol (PBS) may detect a greater amount of allergen concentration than ChemVol extraction buffer (NH_4HCO_3) in ELISA test, because of its influence in the extraction efficacy. However, when PBS was used with ChemVol samples, the allergen

concentration was greater than with NH_4HCO_3 , but still do not approach the sufficient to the amount of allergens detected with Cyclone protocol, both in grasses and in olive.

Daily aeroallergen concentrations as recorded by the two samplers progressed in a similar manner over the pollen season. No significant aeroallergen concentrations were detected here outside the pollination period, although they have been reported by other authors (Viander and Koivikko 1978; Rantio-Lehtimäki et al. 1994; Sánchez-Mesa et al. 2005a; D'Amato et al. 2007; Rodríguez-Rajo et al. 2011). Low aeroallergen levels were recorded before and after the pollen season, particularly for Ole e 1 (Plaza et al. 2016a), higher levels being detected with the Cyclone sampler.

Comparison of aeroallergen with airborne pollen concentrations revealed significant correlations for both Ole e 1 and Phl p 5, but the significance of the correlation tended to be greater with the ChemVol sampler. The ChemVol sampler is probably more efficient at capturing pollen on the solid impacting substrate; however, the Cyclone sampler probably captures both pollen and free allergens in vials.

Fig. 3 Phl p 5 allergen versus Poaceae pollen collected by Cyclone (CY) and ChemVol (CH) samplers in 2012 and 2014

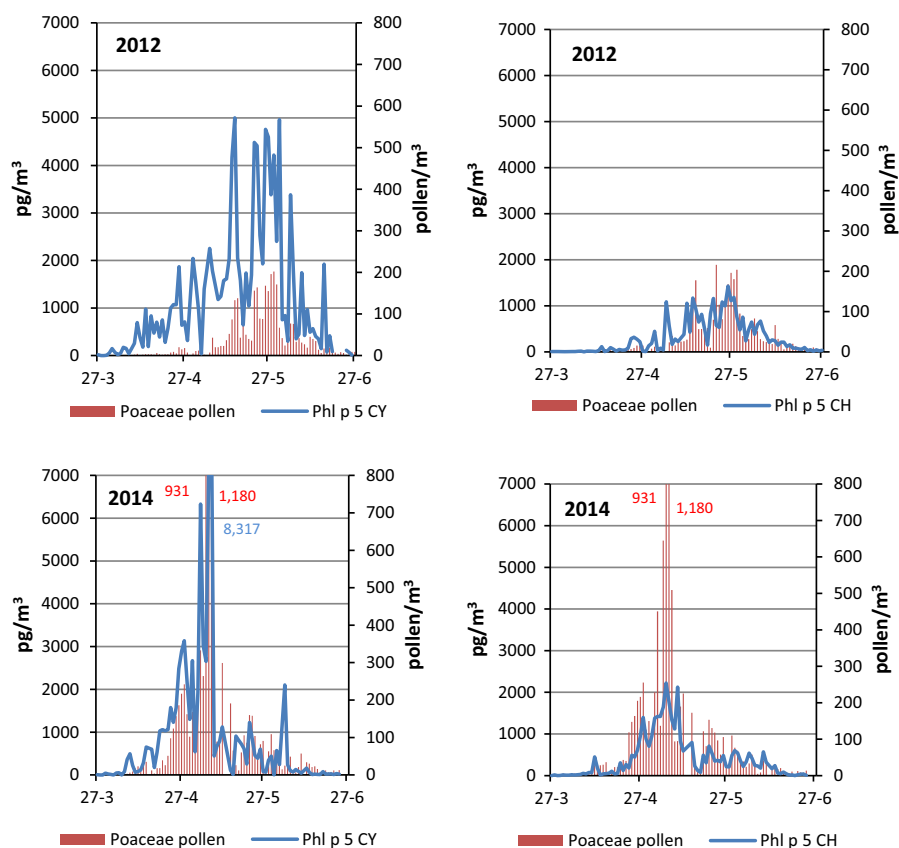


Table 4 Spearman correlations between Poaceae pollen concentrations, airborne allergen Phl p 5 levels and weather variables, and between Olea pollen concentrations, airborne allergen Ole e 1 levels and weather variables

	Pollen/m ³	MT	mT	AT	P	H	W
<i>Olea e 1</i>							
2012							
ChemVol	.867**	.468**	.635**	.613**	-.234*	-.210*	.374**
Cyclone	.866**	.223*	.454**	.492**	-.145	-.108	.549**
Pollen		.414**	.588**	.600**	-.268**	-.284**	.458**
2014							
ChemVol	.928**	.253*	-.118	.133	-.437**	-.374**	-.420**
Cyclone	.841**	.277*	.004	.194	-.323*	-.385**	-.257*
Pollen		.350**	.047	.285**	-.397**	-.497**	-.243*
<i>Phl p 5</i>							
2012							
ChemVol	.805**	.177	.457**	.474**	-.237*	-.204*	.441**
Cyclone	.692**	-.033	.320**	.359**	-.156	-.205	.454**
Pollen		.356**	.585**	.595**	-.380**	-.360**	.388**
2014							
ChemVol	.901**	.428**	.016	.353**	-.388**	-.610**	-.286**
Cyclone	.730**	.170	-.198	.045	-.260*	-.289**	-.329**
Pollen		.373**	-.048	.309**	-.386**	-.557**	-.246*

MT maximum temperature, mT Minimum temperature, AT mean temperature, P rainfall, H relative humidity, W wind speed

* Correlation is significant at the .05 level (2-tailed)

** Correlation is significant at the .01 level (2-tailed)

A marked difference between samplers was observed for Allergy Potency (AP) (allergen vs. pollen) recorded during both study years. Similar findings have been reported for Poaceae by other authors (Jochner et al. 2015; Buters et al. 2015) using the ChemVol sampler. Buters et al. (2015), using this sampler in Córdoba, recorded allergy potencies of 4.15 pg/pollen in 2010—a figure similar to the 8.85 pg/pollen recorded here in 2012—and 3.83 pg/pollen in 2014. However, higher values were obtained here using the Cyclone sampler: 32.19 pg/pollen in 2012 and 8.56 in 2014.

In general, differences between aeroallergen concentrations and pollen concentrations are due to their different sources and reflect different responses to external events. In addition, research that there are more aeroallergens *per* pollen during shorter flowering seasons, as a strategy to ensure pollination (Plaza et al. 2016b). Allergy potency values for Ole e 1 differed both between samplers and between years. Inter-sampler differences were much less marked in 2014, a wetter and colder year.

Results showed a clear correlation between weather variables and both airborne pollen and aeroallergen concentrations. General speaking, significant positive correlations were observed with minimum temperature during the warmer and drier year (2012) and with maximum temperature during the cooler year (2014); a negative significant correlation was observed with rainfall and relative humidity. However, correlations with aeroallergen concentrations measured using the ChemVol sampler were generally higher than with the Cyclone sampler. Galán et al. (2013) and Brito et al. (2011) report that temperature correlates more strongly with allergen concentrations than with pollen concentrations. In the present study, this was only the case for Ole e 1 in 2012 and for Phl p 5 in 2014, suggesting that allergen concentrations are not always more sensitive than pollen concentrations to weather parameters. Correlation with wind speed was significant for both samplers, but significance was greater with the Cyclone sampler, except for *Olea* in 2014. This could be due to the downwind-orienting vane. This could also be one of the best justifications for the difference in collecting allergens between the two methods. Differences were observed in the strength of the correlation between both samplers and wind speed in 2012 and 2014. The significant negative correlation

recorded in 2014 probably confirms the dispersal ability of strong winds (Plaza et al. 2016b).

5 Conclusions

The results of this study suggest that both samplers are appropriate for aeroallergen detection, displaying a significant correlation with airborne pollen concentrations. Qualitatively both methods show the same results, both in seasonal timing and in pollen potency oscillation. However, variations were observed due to differences in sampler design, sampling procedure and allergen extraction. The use of one or another sampler will depend on the purpose of the study.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Alcázar, P., Galán, C., Torres, C., & Domínguez-Vilches, E. (2015). Detection of airborne allergen (Pla a 1) in relation to *Platanus* pollen in Córdoba, South Spain. *Annals of Agricultural and Environmental Medicine*, 22(1), 96–101.
- Andersen, A. A. (1958). New sampler for the collection, sizing and enumeration of viable airborne particles. *Journal of Bacteriology*, 76, 471–484.
- Arilla, M. C., Eraso, E., Ibarrola, I., Algorta, J., Martínez, A., & Asturias, J. A. (2002). Monoclonal antibody-based method for measuring olive pollen major allergen Ole e 1. *Annals of Allergy, Asthma & Immunology*, 89(1), 83–89.
- Arilla, M. C., González-Rioja, R., Ibarrola, I., Mir, A., Monteseirin, J., Conde, J., et al. (2006). A sensitive monoclonal antibody-based enzyme-linked immunosorbent assay to quantify *Parietaria judaica* major allergens, Par j 1 and Par j 2. *Clinical and Experimental Allergy*, 36, 87–93.
- Arilla, M. C., Ibarrola, I., Mir, A., Monteseirin, J., Conde, J., Martínez, A., et al. (2005). Development of a sandwich-type ELISA for measuring Pla a 1, the major allergen of *Platanus acerifolia* pollen. *International Archives of Allergy and Immunology*, 138(2), 127–133.
- Beggs, P. J. (1998). Pollen and pollen antigen as triggers of asthma: What to measure? *Atmospheric Environment*, 32(10), 1777–1783.

- Brito, F. F., Gimeno, P. M., Carnes, J., Martín, R., Fernández-Caldas, E., Lara, P., et al. (2011). *Olea europaea* pollen counts and aeroallergen levels predict clinical symptoms in patients allergic to olive pollen. *Annals of Allergy, Asthma & Immunology*, 106(2), 146–152.
- Buters, J. T., Kasche, A., Weichenmeier, I., Schober, W., Klaus, S., Traidl-Hoffmann, C., et al. (2008). Year-to-year variation in release of bet v 1 allergen from Birch Pollen: Evidence for geographical differences between West and South Germany. *International Archives of Allergy and Immunology*, 2008(145), 122–130. doi:10.1159/000108137.
- Buters, J., Prank, M., Sofiev, M., Pusch, G., Albertini, R., Annesi-Maesano, I., et al. (2015). Variation of the group 5 grass pollen allergen content of airborne pollen in relation to geographic location and time in season. *Journal of Allergy and Clinical Immunology*, 136(1), 87–95.
- Buters, J., Thibaudon, M., Smith, M., Kennedy, R., Rantio-Lehtimäki, A., Albertini, R., et al. (2012). Release of Bet v 1 from birch pollen from 5 European countries. Results from the HIALINE study. *Atmospheric Environment*, 55, 496–505.
- Buters, J. T., Weichenmeier, I., Ochs, S., Pusch, G., Kreyling, W., Boere, A. J., et al. (2010). The allergen Bet v 1 in fractions of ambient air deviates from birch pollen counts. *Allergy*, 65(7), 850–858.
- Cebrino, J., de la Cruz, S. P., Barasona, M. J., Alcázar, P., Moreno, C., Domínguez-Vilches, E., et al. (2017). Airborne pollen in Córdoba City (Spain) and its implications for pollen allergy. *Aerobiologia*. doi:10.1007/s10453-016-9469-8.
- Cebrino, J., Galán, C., & Domínguez-Vilches, E. (2016). Aerobiological and phenological study of the main Poaceae species in Córdoba City (Spain) and the surrounding hills. *Aerobiologia*, 32, 595–606.
- Cecchi, L. (2013). From Pollen count to pollen potency: The molecular era of aerobiology. *European Respiratory Journal*, 42, 898–900.
- Cecchi, L., D'Amato, G., Ayres, J. G., Galan, C., Forastiere, F., Forsberg, B., et al. (2010). Projections of the effects of climate change on allergic asthma: The contribution of aerobiology. *Allergy*, 65(9), 1073–1081.
- D'Amato, G. (2001). Airborne paucimicronic allergen-carrying particles and seasonal respiratory allergy. *Allergy*, 56, 1109–1111.
- D'Amato, G., Cecchi, L., Bonini, S., Nunes, C., Annesi-Maesano, I., Behrendt, H., et al. (2007). Allergenic pollen and pollen allergy in Europe. *Allergy*, 62(9), 976–990.
- D'Amato, G., De Palma, R., Verga, A., Martucci, P., Liccardi, G., & Lobefalo, G. (1991). Antigenic activity of nonpollen parts (leaves and stems) of allergenic plants (*Parietaria judaica* and *Dactylis glomerata*). *Annals of Allergy, Asthma & Immunology*, 67(4), 421–424.
- De Linares, C., Díaz de la Guardia, C., Nieto Lugalde, D., & Alba, F. (2017). Airborne study of grass allergen (Lol p 1) in different-sized particles. *International Archives of Allergy and Immunology*, 152(1), 49–57.
- De Linares, C., Nieto-Lugalde, D., Alba, F., Díaz de la Guardia, C., Galán, C., & Trigo, M. M. (2007). Detection of airborne allergen (Ole e 1) in relation to *Olea europaea* pollen in Spain. *Clinical and Experimental Allergy*, 37(1), 125–132.
- De Weed, N. A., Bhalla, P. L., & Singh, M. B. (2002). Aeroallergens and pollinosis: molecular characteristics of cloned pollen allergens. *Aerobiologia*, 18, 87–106.
- Demokritou, P., Kavouras, I. G., Ferguson, S. T., & Koutrakis, P. (2002). Development of a high volume cascade impactor for toxicological and chemical characterization studies. *Aerosol Science and Technology*, 36(9), 925–933.
- Eder, W., Ege, M. J., & von Mutius, E. (2006). The Asthma Epidemic. *New England Journal of Medicine*, 355(21), 2226–2235.
- Emberlin, J. (1995). Analysis of allergens on airborne particles. Progress and problems. In *European pollenflug-symposium*, vorträge Berichte (pp. 48–62).
- Fernández-Caldas, E., Bandle, E. O., Dunnette, S. L., Swanson, M. C., & Reed, C. E. (1992). Rye grass group allergen content in leaves from seven different grass species. *Grana*, 31, 157–159.
- Fernández-González, D., González-Parrado, Z., Vega Maray, A. M., Valencia-Barrera, R. M., Camazón-Izquierdo, B., De Nuntiis, P., et al. (2010). *Platanus* pollen allergen, Pla a 1: Quantification in the atmosphere and influence on a sensitizing population. *Clinical and Experimental Allergy*, 40(11), 1701–1708.
- Fernández-González, D., Rodríguez Rajo, F., González Parrado, Z., Valencia-Barrera, R., Jato, V., & Moreno Grau, S. (2011). Differences in atmospheric emissions of Poaceae pollen and Lol p 1 allergen. *Aerobiologia*, 27(4), 301–309.
- Galán, C., Antunes, C., Brandao, R., Torres, C., Garcia-Mozo, H., Caeiro, E., et al. (2013). HIALINE working group. Airborne olive pollen counts are not representative of exposure to the major olive allergen Ole e 1. *Allergy*, 68(6), 809–812.
- Galán, C., Cariñanos, P., Alcázar, P., & Domínguez-Vilches, E. (2007). *Manual de Calidad y Gestión de la Red Española de Aerobiología*. Córdoba: Servicio de Publicaciones la University https://www.uco.es/rea/infor_rea/manual_eng.pdf.
- Galán, C., Smith, M., Thibaudon, M., Frenguelli, G., Oteros, J., Gehrig, R., et al. (2014). Pollen monitoring: Minimum requirements and reproducibility of analysis. *Aerobiologia*, 30(4), 385–395.
- Hirst, J. M. (1952). An automatic volumetric spore-trap. *Annals of Applied Biology*, 36, 257–265.
- Jochner, S., Lüpke, M., Laube, J., Weichenmeier, I., Pusch, G., Traidl-Hoffmann, C., et al. (2015). Seasonal variation of birch and grass pollen loads and allergen release at two sites in the German Alps. *Atmospheric Environment*, 122, 83–93.
- León-Ruiz, E., Alcázar, P., Domínguez-Vilchez, E., & Galán, C. (2011). Study of Poaceae phenology in a Mediterranean climate. Which species contribute most to airborne pollen counts? *Aerobiologia*, 27, 37–50.
- Levetin, E. (2004). Methods for Aeroallergen Sampling. *Current Allergy and Asthma Reports*, 4, 376–383.
- Mandrioli, P., Comtois, P., Domínguez Vilches, E., Galán, C., Isard, S., & Syzdek, L. (1998). Sampling: Principles and techniques. In Mandrioli et al. (eds.) *Methods in aerobiology* (pp. 47–112, Pitagora Ed.). Bologna.
- Martínez-Bracero, M., Alcázar, P., Díaz de la Guardia, C., González-Minero, F. J., Ruiz, M., Trigo, M., et al. (2015).

- Pollen calendars: A guide to common airborne pollen in Andalusia. *Aerobiologia*, 31, 549–557.
- Moreno-Grau, S., Elvira-Rendueles, B., Moreno, J., García-Sánchez, A., Vergara, N., Asturias, J. A., et al. (2006). Correlation between *Olea europaea* and *Parietaria judaica* pollen counts and quantification of their major allergens Ole e 1 and Par j 1 e Par j 2. *Annals of Allergy, Asthma & Immunology*, 96(6), 858–864.
- Plaza, M. P., Alcázar, P., & Galán, C. (2016a). Correlation between airborne *Olea europaea* pollen concentrations and levels of the major allergen Ole e 1 in Córdoba, Spain, 2012–2014. *International Journal of Biometeorology*, 60(12), 1841–1847.
- Plaza, M. P., Alcázar, P., Hernández-Ceballos, M. A., & Galán, C. (2016b). Mismatch in aeroallergens and airborne grass pollen concentrations. *Atmospheric Environment*, 144, 361–369.
- Rantio-Lehtimäki, A., Viander, M., & Koivikko, A. (1994). Airborne birch pollen antigens in different particle sizes. *Clinical and Experimental Allergy*, 24(1), 23–28.
- Rodríguez-Rajo, F. J., Jato, V., González-Parrado, Z., Elvira-Rendueles, B., Moreno-Grau, S., Vega-Maray, A., et al. (2011). The combination of airborne pollen and allergen quantification to reliably assess the real pollinosis risk in different bioclimatic areas. *Aerobiologia*, 27(1), 1–12.
- Sánchez-Mesa, J. A., Brandao, R., Lopes, L., & Galán, C. (2005a). Correlation between pollen counts and symptoms in two different areas of the Iberian Peninsula: Córdoba (Spain) and Évora (Portugal). *Journal of Investigational Allergology and Clinical Immunology*, 15, 112–116.
- Sánchez-Mesa, J. A., Serrano, P., Cariñanos, P., Prieto-Baena, J. C., Moreno, C., Guerra, F., et al. (2005b). Pollen allergy in Córdoba city: Frequency of sensitization and relation with antihistamine sales. *Journal of Investigational Allergology and Clinical Immunology*, 15, 50–56.
- Scheifinger, H., Belmonte, J., Buters, J., Celenk, S., Damialis, A., Dechamp, C. et al. (2013). Monitoring, modelling and forecasting of the pollen season. In: M. Sofiev & K.-C. Bergmann (Eds.), *Allergenic pollen* (pp. 71–126). Netherlands: Springer. doi:10.1007/978-94-007-4881-1_4.
- Takahashi, Y., Ohashi, T., Nagoya, T., Sakaguchi, M., Yasueda, H., & Nitta, H. (2001). Possibility of real-time measurement of an airborne *Cryptomeria japonica* pollen allergen based on the principle of surface Plasmon resonance. *Aerobiologia*, 17, 313–318.
- Velasco-Jiménez, M. J., Alcázar, P., Domínguez-Vilches, E., & Galán, C. (2013). Comparative study of airborne pollen counts located in different areas of the city of Córdoba (south-western Spain). *Aerobiologia*, 29, 113–120.
- Viander, M., & Koivikko, A. (1978). The seasonal symptoms of hyposensitized and untreated hay fever patients in relation to birch pollen counts: Correlations with nasal sensitivity, prick tests and RAST. *Clinical and Experimental Allergy*, 8(4), 387–396.