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Chapter 4

Involvement of Climatic Factors in the Allergen Expression in Olive Pollen

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/50077

1. Introduction

Olive allergen concentrations in the pollen grain are critical in allergic response of atopic patients. Existing evidence shows that climatic factors can influence pollen allergen content. To date, the influences of temperature and precipitations over the content of birch and olive pollens in their respective major allergens have been analyzed. Bet v 1, the major allergen of birch pollen, increased its expression (Buters et al. 2008) and presented a higher allergenicity (Ahlholm et al. 1998) while temperatures were elevated. Differently, the content in the major olive pollen allergen Ole e 1 showed no apparent correlation with either temperatures or precipitations (Fernández Caldas et al. 2007). However, a positive correlation between total allergenicity and rainfall occurring in winter months was found. This correlation was not analyzed independently for each pollen allergen.

Our aim was to extend the observations carried out in olive pollen, by analyzing the effects of climate parameters over the expression of four olive pollen allergens. Using a recently developed multiplex western blotting system for the assessment of allergenic molecules (Morales et al. 2012), we have simultaneously detected and quantified two major (Ole e 1 and Ole e 9) and two minor allergens (Ole e 2 and Ole e 5) in the pollen extracts from seven olive cultivars collected along 4-7 consecutive years. The considered climatic variables included temperature, precipitation, number of rain days and humidity. Data were provided by the Spanish network for the temporal observation of ecosystems (REDOTE). Correlations between the allergen contents (both individually and all inclusive) and climate variables were studied by applying Spearman correlation tests. Results showed significant variations in the expression of the four allergens in the seven cultivars throughout the years of the analysis. All these positive correlations corresponded exactly to the period of time starting the winter prior to each flowering period to the end of period (this is, December from the previous year to June).

These results are discussed as regard to their putative incidence in the development of symptoms by patients, the requirements for medical assistance and the rates of admission into clinical centres and hospitals. Agronomical implications in olive sexual reproduction, including fruit setting and fruit production, are also discussed.

2. Materials and methods

2.1. Pollen samples

Olea europaea L. pollen samples were obtained during May and June of 2000-2007 from cultivated trees of the cultivars: 'Arbequina', 'Blanqueta', 'Hojiblanca', 'Manzanilla de Sevilla', 'Picual', 'Verdial de Huévar' and 'Verdial de Vélez' situated at the IFAPA center "Alameda del Obispo" (Andalusian Regional Government, Córdoba, Spain). Pollen samples were collected from numerous branches of at least two trees of each cultivar by shaking flowering shoots inside paper bags. Prior to its storage in liquid nitrogen, the harvested pollen was sieved through a 150 μm mesh in order to eliminate fallen corollas, anthers and other rests. After light microscopy observation, foreign-species pollen was estimated to be <0.1% and other plant parts <0.5% for all the cultivars used.

2.2. Preparation of crude protein extracts and SDS-PAGE

Crude protein extracts were obtained by stirring 1 g of pollen for each cultivar in 10 ml extraction buffer (0.01 M ammonium bicarbonate, pH 8.0, and 2 mM phenylmethylsulfonyl fluoride) for 8 h at 4°C. After centrifugation (2 x 30 minutes at 14,000 rpm at 4°C), the supernatants were filtered through a 0.2 μm filter, and stored in aliquots at –20°C. Protein concentration in the different samples was measured using the Bio-Rad reagent (Bio-Rad, Hercules, CA, USA) and bovine serum albumin (BSA) as standard.

Proteins (30 μg per lane) and Mw1 (New England BioLabs, Ipswich, MA, USA) and Mw2 standards (MBI Fermentas, Vilnius, Lithuania) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 15% gels in a MiniProtean II system (Bio-Rad). The resulting gels were stained with silver nitrate (Rabilloud et al., 1994).

2.3. Immunoblotting

Gels obtained as described above were transferred onto BioTrace® polyvinylidene difluoride (PVDF) membranes (Pall BioSupport, Port Washington, NY, USA) at 100 V for 1.5 hours using a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad). Prior to the treatment with antibodies, the membranes were blocked with TBST buffer (Tris buffered saline: TBS + 0.3% v/v Tween 20) + 10% w/v dried skimmed milk. For membrane probing, a recently developed multiplex Western blotting system for the assessment of allergenic molecules (Morales et al. 2012) has been used. Briefly, the simultaneous detection of four olive pollen allergens (Ole e 1, Ole e 2, Ole e 5 and Ole e 9) on a single blot using a monoclonal antibody from mouse and three polyclonal antibodies raised in rabbit is carried out. We utilized

unconjugated Fab antibody fragments for blocking rabbit primary antibodies, and fluorescence-based detection. These changes allowed an accurate and reliable comparative quantitation of these allergens among pollen protein samples.

2.4. Absolute and relative quantitation of allergens

Imaging was carried out with a Pharos FX Plus Molecular Imager (Bio-Rad) using the Quantity One v4.6.2 software (Bio-Rad). The intensity of each fluorescent band was calculated using the quantitation tools of the Quantity One v4.6.2 software.

2.5. Climate parameters

Climate parameters were obtained from the weather tracking station situated at Córdoba airport (Spain) during the years 2000 to 2007. Information was extracted from the Spanish long term ecological research network (REDOTE).

2.6. Statistical analysis

Continuous variables were subjected to a study of normality distribution by using the Kolmorov-Smirnov test. Associations between continuous variables (non-normal distribution) were described by analysis of bivariate Spearman correlation (two-tailed). Significant correlation was considered $P<0.05$.

3. Results

3.1. Multiplex determination of year-to-year allergen expression in seven olive cultivars

Figure 1A shows the protein profiles of the extracts analyzed after SDS-PAGE and silver staining. The different panels correspond to the cultivars analyzed between the years 2000 and 2007. Bands were observed in the range of 15 to 75 kDa. Clear quantitative differences were distinguished, from which the most conspicuous were those in the bands of 20 kDa and 18.4 kDa among de different years, with the exception of cultivars 'Arbequina', 'Verdial de Huévar' and 'Hojiblanca', in which the Ole e 1 bands were not well identified after silver staining. The remaining allergens studied were not identified after silver staining.

Allergenic profiles were assessed by multiplex immunotransference (Figure 1B). The cultivars 'Arbequina' and 'Hojiblanca' displayed two Ole e 1 forms (20 kDa and 18.4 kDa) in contrast with the remaining cultivars, were the three major forms of Ole e 1 (22 kDa, 20 kDa and 18.4 kDa) were distinguished. Differences in the expression of this allergen depending on the year of pollen collection can be easily noticed.

Ole e 2 was visualized in all cultivars in the form of two bands with sizes of 15.1 kDa and 14 kDa, with the exception of the 'Verdial de Vélez' cultivar, in which only the 15.1 kDa band was present. As described for Ole e 1, the presence of noticeable differences in the

expression of the Ole e 2 allergen, depending the year of pollen collection can be observed in the corresponding multiplex immunoblots.

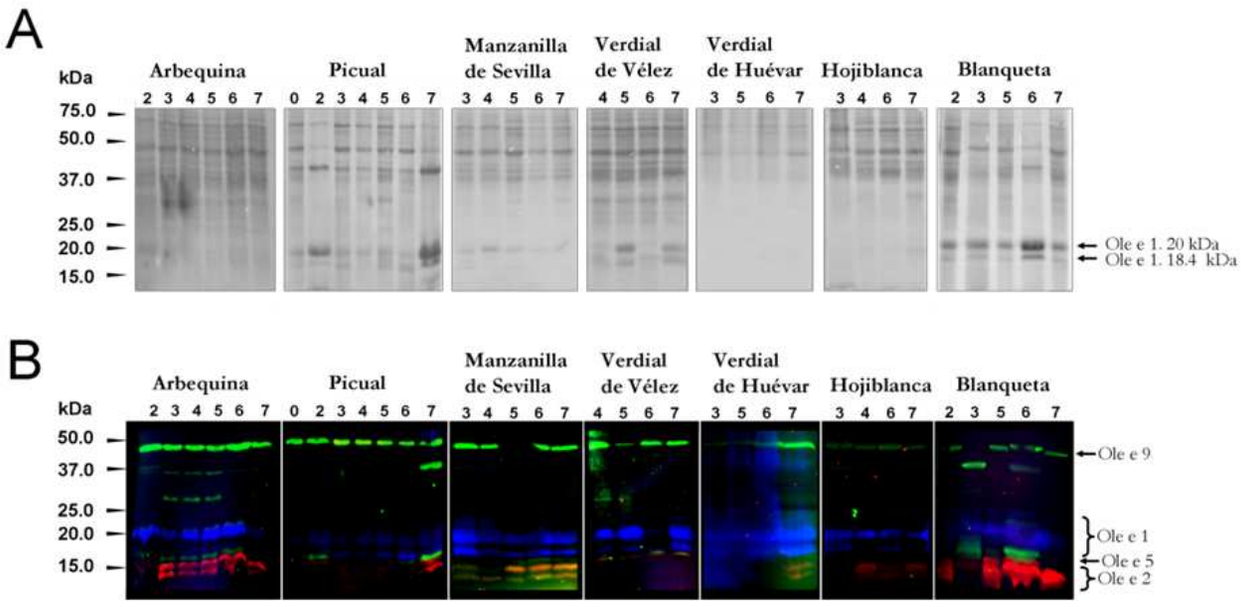

Figure 1. A) Silver staining of total protein extracts obtained from pollen corresponding to the seven cultivars analyzed trhoughout the different years. B) Multiplex detection of the allergens Ole e 1, Ole e 2, Ole e 5 and Ole e 9 in the same extracts described in panel A. Thirty micrograms of total protein were loaded in each lane. Lane numbers (0-7) correspond to the years analyzed (2000-2007). Molecular weight standards are displayed on the left.

Ole e 5 allergen can be observed in the cultivars analyzed. However, only a weak signal was detected in the cultivars 'Hojiblanca' and 'Verdial de Huévar'. Year-to-year differences in the expression of this allergen are clearly observed in 'Picual' and 'Blanqueta' cultivars.

Finally, Ole e 9 was expressed in all cultivars analyzed. Year-to-year differences in their expression are observed as well, with the sharpest differences in the cultivars 'Blanqueta' and 'Manzanilla de Sevilla'.

3.2. Involvement of climatic factors in the expression of Ole e 1 allergen

Correlation between the data corresponding to the major climatic factors and the expression of the allergens Ole e 1, Ole e 2, Ole e 5 y Ole e 9 was individually assessed for each cultivar by means of Spearman correlation analyses. The analysis of the correlation between the levels of the Ole e 1 and these climatic parameters is shown in Table 1. The presence of a statistically significant positive correlation between the expression of the different Ole e 1 forms (either individually or their added values) and the accumulated yearly average temperatures (the addition of the monthly average temperatures during those months prior to the blooming period -July of the prior year to June of the current year-) was determined for the cultivar 'Blanqueta'. Total levels of the Ole e 1 allergen correlated positively with the monthly average temperatures during the winter period (for the cultivar 'Verdial de Huévar') or the spring period (cultivars 'Blanqueta', 'Hojiblanca').

Cultivar	Climatic factor		Ole e 1 22 kDa	Ole e 1 20 kDa	Ole e 1 18.4 kDa	All Ole e 1 bands
Arbequina	Yearly AT accumulated (°C)	R	-----		.65	
		P			.156	
Blanqueta	Yearly AT accumulated (°C)	R	.9*	1**		.9*
		P	.037	<0.001		.037
	AT April (°C)	R		.9*		
		P		.037		
	AT May (°C)	R		.9*		
		P		.037		
	mT May (°C)	R	.9*	1**		0.9*
		P	.037	<0.001		.037
Hojiblanca	AT March (°C)	R	-----		1**	1**
		P			<0.001	<0.001
	mT March (°C)	R	-----		1**	1**
		P			<0.001	<0.001
	Accumulated precipitation winter (January-March) (mm)	R	-----	1**		
		P		<0.001		
	precipitation February (mm)	R	-----	1**		
		P		<0.001		
Picual	AT April (°C)	R			.643	
		P			.119	
	mT April (°C)	R	-----		.829*	
		P			.021	
	MT April (°C)	R	-----		.757*	
		P			.049	
Verdial de Vélez	No. days of rain in August	R	1**		1**	1**
		P	<0.001		<0.001	<0.001
Verdial de Huévar	AT February (°C)	R		1**	1**	1**
		P		<0.001	<0.001	<0.001
	Accumulated precipitation (January-May) (mm)	R		1**	1**	1**
		P		<0.001	<0.001	<0.001
	No. days of rain in February	R	.949	.949	.949	.949
		P	.05	.05	.05	.05
	No. days of rain in April	R	1**			
		P	<0.001			
	Accumulated mRH (January-May) (%)	R		1**	1**	1**
		P		<0.001	<0.001	<0.001
	mRH January (%)	R		1**	1**	1**
		P		<0.001	<0.001	<0.001

Cultivar	Climatic factor		Ole e 1 22 kDa	Ole e 1 20 kDa	Ole e 1 18.4 kDa	All Ole e 1 bands
	mRH February (%)	R	1**			
		P	<0.001			
	mRH April (%)	R		1**	1**	1**
		P		<0.001	<0.001	<0.001
	mRH May (%)	R		1**	1**	1**
		P		<0.001	<0.001	<0.001
Manzanilla de Sevilla	AT March (°C)	R			.8	
		P			.104	
	AT March (°C)	R			.7	
		P			.108	
	mT March (°C)	R		.9*		
		P		.037		
	MT May (°C)	R		.9*		
		P		.037		
	Accumulated yearly precipitation (mm)	R	.9*	1**		0.9*
		P	.037	.001		.037
	precipitation July (mm)	R		.894		.894
		P		.04		.04
	precipitation September (mm)	R	1**			
		P	<0.001			
	precipitation November (mm)	R	.9*	.9*		.9*
		P	.03	.037		.037
	precipitation April (mm)	R		.9*		.9*
		P		.037		.037
	No. days of rain in January	R		.9*		.9*
		P		.037		.037
	No. days of rain in April	R		.9*		.9*
		P		.037		.037
	Accumulated yearly mRH (%)	R	1**			
		P	<0.001			
	mRH December (%)	R	.9*			
		P	.037			
	mRH February (%)	R		.9*		.9*
		P		.037		.037

AT: average temperature. mT: minimum temperature. MT: maximum temperature. mRH: monthly relative humidity

Table 1. Analysis of bivariate Spearman correlation (two-tailed) between the expression of the different Ole e 1 forms both individually and jointly (CNT*mm^2) and different climatic factors. R: Spearman correlation coefficient. * Statistically significant correlation P<0.05; ** P<0.01.

Moreover, statistically significant positive correlations were observed between Ole e 1 expression and minimum temperatures (cultivars 'Blanqueta', 'Hojiblanca', 'Picual' and 'Manzanilla de Sevilla') or maximum temperatures (cultivars 'Picual' and 'Manzanilla de Sevilla') during the spring period.

Total expression of Ole e 1 correlated positively with the added monthly precipitation occurred during the winter and spring period (cultivars 'Hojiblanca' and 'Verdial de Huévar'), as well as with the total precipitation occurred during the months prior to flowering (cultivar 'Manzanilla de Sevilla'). Positive correlations are also observed between total levels of Ole e 1 allergen and precipitation, number of days of rain, and the monthly average of relative humidity during the months prior to the blooming season.

3.3. Involvement of climatic factors in the expression of Ole e 2 allergen

Table 2 displays the observed correlations between Ole e 2 and different climatic factors.

Cultivar	Climatic factor		Ole e 2 15.1 kDa	Ole e 2 14 kDa	All Ole e 2 bands
Arbequina	AT June (°C)	R	.886*		.886*
		P	.019		.019
	MT June (°C)	R	.886*		.886*
		P	.019		.019
	Accumulated precipitation winter (January-March) (mm)	R		.943**	
		P		.005	
	precipitation February (mm)	R		.943**	
		P		.005	
Hojiblanca	AT February (°C)	R	1**		
		P	<0.001		
	mT April (°C)	R			.829*
		P			.021
	MT April (°C)	R			.757*
		P			.049
	Accumulated mRH (January-May) (%)	R	1**		
		P	<0.001		
	mRH January (%)	R	1**		
		P	<0.001		
	mRH May (%)	R	1**		
		P	<0.001		
Verdial de Vélez	mRH December (%)	R			1**
		P			<0.001
Verdial de Huévar	AT January (°C)	R		1**	
		P		<0.001	
	precipitation June (mm)	R		.775*	
		P		.041	

	Accumulated No. of raining days (January-May)	R P		1** <0.001	
	No. days of rain in February	R P	.949 .05	.949 .05	.949 .05
	No. days of rain in April	R P	1** <0.001		1** <0.001
	Accumulated mRH (January-May) (%)	R P		1** <0.001	
	mRH February (%)	R P	1** <0.001		1** <0.001
	mRH April (%)	R P	1** <0.001		
	mRH May (%)	R P	1** <0.001		
Manzanilla de Sevilla	precipitation June (mm)	R P	.949* .014	.949* .014	.949* .014
	No. days of rain in June	R P	.872 .05	.872 .05	.872 .05

Table 2. Analysis of bivariate Spearman correlation (two-tailed) between the expression of the different Ole e 2 forms both individually and jointly (CNT*mm^2) and different climatic factors. R: Spearman correlation coefficient. * Statistically significant correlation P<0.05; ** P<0.01. AT: average temperature. mT: minimum temperature. MT: maximum temperature. mRH: monthly relative humidity.

The expression levels of the bands corresponding to the Ole e 2 allergen (Table 2) and/or their addition, correlated positively with the average temperatures for particular months during the winter period (cultivars 'Hojiblanca' and 'Verdial de Huévar') or spring (cultivar 'Arbequina'). Statistically significant correlations were also observed between the expression of Ole e 2 and the maximum/minimum temperatures over the months corresponding to the spring (cultivars 'Arbequina' and 'Hojiblanca').

The expression of one of the Ole e 2 form is positively correlated to the accumulated monthly precipitation occurred during the winter months for the cultivar 'Arbequina', as well as to the precipitation occurred in particular months during the winter and spring (cultivars 'Arbequina', 'Verdial de Huévar' and 'Manzanilla de Sevilla'). The Ole e 2 form with the higher molecular weight (15.1 kDa) also presented correlation to the accumulated monthly relative humidity during the months of winter and spring (cultivars 'Hojiblanca' and 'Verdial de Huévar'). Similarly, positive correlation was detected between the total levels of Ole e 2 and the total number of days of rain over the winter and spring months (cultivar 'Verdial de Huévar').

3.4. Involvement of the climatic factors in the expression of Ole e 5 allergen

As respects to Ole e 5 allergen (Table 3), the presence of a statistically significant positive correlation was determined between the minimum temperature during May, and the precipitation observed during February in the 'Blanqueta' cultivar. In the cultivar 'Verdial

de Vélez', Ole e 5 correlated to the number of days of rain and the relative humidity over the months of February and April.

Cultivar	Climatic factor		Ole e 5
Blanqueta	mT May (°C)	R	.9*
		P	.0187
	precipitation February (mm)	R	.9 *
		P	.0037
Verdial de Vélez	No. days of rain in February	R	1**
		P	<0.001
	No. days of rain in April	R	1**
		P	<0.001
	mRH February (%)	R	1**
		P	<0.001
	mRH April (%)	R	1**
		P	<0.001

Table 3. Analysis of bivariate Spearman correlation (two-tailed) between the expression of Ole e 5 (CNT*mm²) and different climatic factors. R: Spearman correlation coefficient. *Statistically significant correlation P<0.05; ** P<0.01. AT: average temperature. mT: minimum temperature. MT: maximum temperature. mRH: monthly relative humidity.

3.5. Involvement of climatic factors in the expression of Ole e 9 allergen

As regard to the Ole e 9 allergen, its expression correlated positively (Table 4) with the average temperatures during the months of January (cultivars 'Arbequina', and 'Verdial de Huévar') and March ('Hojiblanca'). In the same way, a correlation was demonstrated between Ole e 9 expression and the number of days of rain during March in the cultivars 'Arbequina' and 'Verdial de Vélez'. The average relative humidity also showed a statistically relevant correlation during the months of winter, for the cultivar 'Picual', and specifically for January in the cultivar 'Verdial de Huévar'. In addition, and for this last cultivar 'Verdial de Huévar', the relative humidity presents a positive correlation during the spring months of April and May.

3.6. Several examples of data distributions

We describe next several examples of data distributions corresponding to climatic factors showing a statistically significant correlation with the expression level of the different allergens. Charts represent the allergen levels (represented as band intensities) plotted against climatic factors like temperature (Figure 2), precipitation, relative humidity and number of days of rain (Figure 3). A reference line showing a theoretical absolute linear correlation between the variables was added to the figures.

Cultivar	Climatic factor		Ole e 9
Arbequina	AT January (°C)	R	.829*
		P	.042
	No of days of rain in March	R	.088*
		P	.02
Hojiblanca	AT March (°C)	R	1**
		P	<0.001
	mT March (°C)	R	1**
		P	<0.001
Picual	mRH December (%)	R	.857*
		P	.014
Verdial de Vélez	No. days of rain in March	R	.949
		P	.05
Verdial de Huévar	AT January (°C)	R	1**
		P	<0.001
	No. days of rain in February	R	.949
		P	.05
	mRH January (%)	R	1**
		P	<0.001
	mRH April (%)	R	1**
		P	<0.001
	mRH May (%)	R	1**
		P	<0.001

Table 4. Analysis of bivariate Spearman correlation (two-tailed) between the expression of Ole e 9 (CNT*mm²) and different climatic factors. R: Spearman correlation coefficient. *Statistically significant correlation P<0.05; ** P<0.01. AT: average temperature. mT: minimum temperature. MT: maximum temperature. mRH: monthly relative humidity.

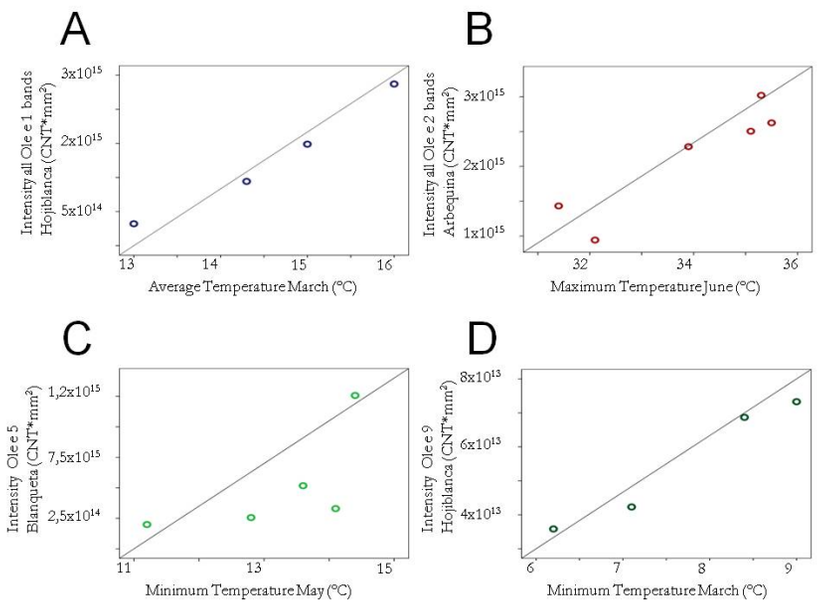

Figure 2. Examples of charts displaying data distribution referring to temperatures plotted against the expression levels of the following allergens: A) Ole e 1, B) Ole e 2, C) Ole e 5 and D) Ole e 9.

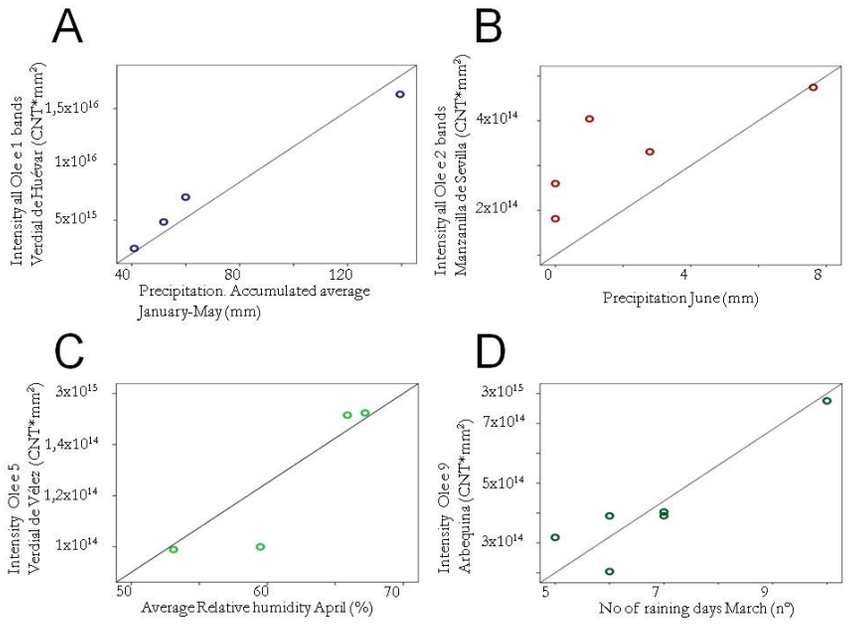

Figure 3. Examples of charts displaying data distribution referring to precipitation, relative humidity and number of days of rain plotted against the expression levels of the following allergens: A) Ole e 1, B) Ole e 2, C) Ole e 5 and D) Ole e 9.

4. Discussion and conclusions

The allergen content of the olive pollen is clearly influenced by the genetic origin of the cultivar analyzed (Hamman Khalifa et al. 2008). Diverse authors have also indicated that climatic factors may have different effects over olive flowering and pollen development (Galan et al. 2001; Orlandi et al. 2010), as well as over olive pollen allergenicity (Fernández-Caldas et al. 2007).

The results presented here demonstrate that changes occur in the expression of four relevant allergens in seven cultivars, and throughout consecutive years. However, results are considered heterogeneous between the different cultivars. The climatic parameters taken into account were temperature, precipitation, number of days of rain or relative humidity during the months prior to the flowering period, which normally corresponds to the months of May and June.

As regard to the temperature, correlations were not observed for a particular month or for the accumulated yearly temperature in all cultivars in a simultaneous way. However, the point correlations detected always corresponded to the winter and summer months (January-June) prior to the flowering period. This can be due to the intrinsic characteristics and requirements of each cultivar, or to the low number of cultivars and years studied, which may limit the statistical power of the analysis.

This results are in good agreement with those described for the birch major pollen allergen Bet v 1 (Buters et al. 2008), where an increase of this allergen was observed in higher temperatures, determined by growth of sampling trees in different climatic regions, over

two consecutive years. Another study suggests a higher allergenic response to the Bet v 1 allergen present in birch pollen extracts grown in high average daily temperatures, in comparison to trees cultivated at lower temperatures (Ahlholm et al. 1998). This assay was carried out by using sera from patients sensitive to this pollen by using immunoblotting experiments. Our results indicate that high temperatures also seem to exert an increase in the expression of allergens in certain olive cultivars.

We may extrapolate that an increase in the average temperatures would bring a parallel increase in pollen allergenic content and allergenicity as a consequence. This could be therefore one of the consequences of the so-called global warming produced by the greenhouse effect (Ahlholm et al. 1998; Cecchi et al. 2010). Moreover, we may speculate that the fact that urban areas are often exposed to temperatures 2 to 8 °C higher that rural areas (Oke 1987) may result in increased olive pollen allergenicity and allergy prevalence in and around the cities, where the olive tree is also present, as it is widely used as an ornamental plant.

Fernández-Caldas et al. (2007) performed a similar study using six olive cultivars, over a period of five consecutive years. This study analyzed the content in the olive pollen major allergen (Ole e 1) only, which did not show statistically significant correlations to the precipitation average, nor to the average temperatures over the winter months prior to flowering. These authors described, however, a positive correlation between overall allergenicity and the average precipitation occurred during the winter months, for all the cultivars analyzed.

Several relevant differences are noticeable between the present study and that of Fernández-Caldas et al. (2007). First, this work combines the information available on four allergens, Ole e 1, Ole e 2, Ole e 5 and Ole e 9, instead of the olive pollen major allergen only. Therefore, the conclusions obtained are more similar to those described by Fernández-Caldas et al. referring to whole olive pollen allergenicity than those referring to Ole e 1 only. On the other hand, the present study uses accumulated data instead of averages for parameters like precipitation and number of monthly/seasonal/yearly days of rain. Moreover, although in some cases, monthly averages of temperature and relative humidity were considered, specific correlations were calculated upon the accumulated data for seasonal/yearly periods.

From an allergenic point of view, the present results suggest that those years with high levels of precipitation and high temperatures during the winter and the spring periods, contribute an enhanced allergenic content of the pollen grains at the end of the spring. We also may postulate that those areas of olive culture which are supplemented by artificial watering may yield a higher allergenicity for olive pollen-sensitized patients. The same would apply to urban areas, where ornamental olive trees are usually watered.

From an agronomic point of view, the described increase in the expression of the allergen Ole e 1 which takes place under increased temperature and precipitation might result in important differences in the reproductive behavior of the plant. Thus, preliminary observations (Morales et al. unpublished), indicated that the enhanced levels of Ole e 1

correlated to increased rates of pollen germination and lower degree of olive self-incompatibility. This hypothesis is also in good agreement with experimental observations made by several authors as regard to the effects of climatic factor on self-pollination and productivity in the olive (Androulakis and Loupassaki 1990, Lavee et al. 2002).

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Acknowledgement

This work was supported by the Spanish Ministry of Science and Innovation (MICINN) (ERDF-cofinanced projects AGL2008-00517, BFU2011-22779 and PIE-200840I186) and the Junta de Andalucía (ERDF-cofinanced projects P2010-CVI5767 and P2010-AGR6274). The authors acknowledge the availability of plant material and the collaboration of the staff of the IFAPA center "Venta del Llano" (Mengíbar, Spain) depending from the Andalusian Regional Government.

5. References

Ahlholm, J.U., Helander, M.L., & Savolainen, J. (1998). Genetic and environmental factors affecting the allergenicity of birch (Betula pubescens ssp. czerepanovii [Orl.] Hamet-ahti) pollen. Clinical and Experimental Allergy, Vol. 28, No. 11, pp. 1384-1388, ISSN 1365-2222

Androulakis, I.I., & Loupassaki, M.H. (1990). Studies on the self-fertility of some cultivars in the area of Crete. *Acta Horticulturae*, Vol. 286, pp. 159-162, ISSN 0567-7572

Buters, J.T., Kasche, A., Weichenmeiera, I., Schober, W., Klaus, S., Traidl-Hoffmann, C., Menzel, A., Huss-Marp, J., Krämer, U., & Behrendt, H. (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 & Immunology*, Vol. 145, No. 2, pp. 122-130, ISSN 1018-2438

Cecchi, L., D'Amato, G., Ayres, J.G., Galan, C., Forastiere, F., Forsberg, B., Gerritsen, J., Nunes, C., Behrendt, H., Akdis, C., Dahl, R., & Annesi-Maesano, I. (2010). Projections of the effects of climate change on allergic asthma: the contribution of aerobiology. *Allergy*, Vol. 65, No. 9, pp. 1073-1081, ISSN 0105-4538

Fernández-Caldas, E., Carnés, J., Iraola, V., & Casanovas, M. (2007). Comparison of the allergenicity and Ole e 1 content of 6 varieties of Olea euroapea pollen collected during 5 consecutive years. *Annals of Allergy, Asthma & Immunology*, Vol. 98, No. 5, pp. 464-470, ISSN 1081-1206

Galan, C., Cariñanos, P., Garcia-Mazo, H., Alcazar, P., & Dominguez-Vilches, E. (2001). Model for forecasting *Olea europaea* L. airborne pollen in South-West Andalusia, Spain. *International Journal of Biometeorology*, Vol. 45, No. 2, pp. 59-63, ISSN 0020-7128

Hamman Khalifa, A.M., Castro, A.J., Rodríguez García, M.I., & Alché, J.D (2008). Olive cultivar origin is a major cause of polymorphism for Ole e 1 pollen allergen. *BMC Plant Biology*, Vol. 8, No. 10, ISSN 1471-2229

Lavee, S., Taryan, J., Levin, J., & Haskal, A. (2002). Importancia de la polinización cruzada en distintas variedades de olivo cultivadas en olivares intensivos de regadío. *Olivae: revista oficial del Consejo Oleícola Internacional*, Vol. 91, pp. 25-36, ISSN 0255-996X

Oke, T.R. (1987). Boundary Layer Climates. 2nd. Edition. London: Routledge. 435 pp.

Orlandi, F., Sgromo, C., Bonofiglio, T., Ruga, L., Romano, B., & Fornaciari, M. (2010). Spring Influences on Olive Flowering and Threshold Temperatures Related to Reproductive Structure Formation. *HortScience*, Vol. 45, No. 7, pp. 1052-1057, ISSN: 0018-5345

Morales, S., Castro, A.J., Jiménez-López, J.C., Florido, F., Rodríguez-García, M.I., & Alché J.D. (2012). A novel multiplex method for the simultaneous detection and relative quantification of pollen allergens. *Electrophoresis*, Vol. 33, pp. 1-8, DOI 10.1002/elps.201100667, ISSN: 1522-2683

Rabilloud, T., Vuillard, L., Gilly, C., & Lawrence, J.J. (1994). Silver-staining of proteins in polyacrylamide gels: a general overview. *Cellular and Molecular Biology (Noisy-le-Grand, France)*, Vol. 40, No. 1, pp. 57-75, ISSN 1165-158X