

Short communication

An occupational respiratory allergy caused by *Sinapis alba* pollen in olive farmers

Background: *Sinapis alba* (white mustard) is a entomophilic species included in the Brassicaceae family. To date it has not been related to allergic sensitization or clinical respiratory disease.

Methods: Twelve olive orchard workers had a history of rhinitis and/or bronchial asthma that occurred during control weed management and/or harvest, from January to March. They underwent skin prick tests (SPT) with *S. alba* pollen extract and a standard battery of aeroallergens. *Sinapis alba* pollen extract was prepared for performing quantitative skin tests, enzyme allergosorbent test and nasal challenge test (NCT). A portable monitoring station and an urban volumetric Hirst-type spore trap were used for the aerobiological study.

Results: Eleven patients suffered from rhinitis and bronchial asthma and one had only from rhinitis. All patients were sensitized to *S. alba* pollen extract, and they showed a positive NCT response. In the urban aerobiologic monitoring station the amount of *S. alba* pollen only exceptionally reached peaks of 21 grains/m³, whereas in the work environment peaks of 1801 grains/m³ were detected between 15 February and 7 April.

Conclusions: We demonstrate the existence of a new occupational allergen for olive farmers: *S. alba* pollen. We point out the importance of perform aerobiological sampling within the occupational environment for the detection and quantification of the allergenic source.

**J. L. Anguita¹, L. Palacios¹,
L. Ruiz-Valenzuela², B. Bartolomé³,
M^a. J. López-Urbano¹, B. Sáenz de
San Pedro¹, E. Cano², J. Quiralte¹**

¹Allergology Unit, Complejo Hospitalario de Jaén, Jaén; ²Department of Botany, Science Faculty, University of Jaén, Jaén; ³Bial – Aristegui, Alameda Urquijo, Bilbao, Spain

Key words: bronchial asthma, occupational respiratory allergy, olive farmers, rhinitis, *Sinapis alba* pollen.

Dr J. L. Anguita
Unidad de Alergología
Hospital Universitario Médico Quirúrgico
Complejo Hospitalario de Jaén
Avenida del Ejército Español 10
23007 Jaén
Spain

Accepted for publication 27 November 2006

Olive farming is a key element of agriculture in Andalucía, a Spanish region. There are a lot of different species of annual weeds which infest the olive orchard of Jaén, as it happens with some species of Brassicaceae family, like *Sinapis alba* (white mustard), a 20–100 cm weed with intensely yellow flowers (1). This species is very widespread in southern Europe and northern Africa, where it grows wild together with a great diversity of crops. It is also cultivated for obtaining white mustard. To date, it has not been related to allergic sensitization or clinical respiratory disease.

Weed control and harvesting are two critical activities in olive crop care. Both maneuvers, made by olive farmers, imply a narrow contact with olive orchard, at the time during which *S. alba* flowers (late winter to early spring). Like other Brassicaceae species, their pollen is prolate or subspheroidal, small to medium sized, trizonocolporate, isopolar, and radioisometric with a reticulate surface (2).

Patients and methods

Patients

Twelve patients fulfilled the following criteria: (i) they were olive orchard workers; and (ii) they had a history of rhinitis and/or bronchial asthma that occurred exclusively during control weed management and/or harvest during late winter (January–March). They showed sensitization without clinical relevance to another plants, which do not flower at the same time as *S. alba*.

The clinical data of the patients included age, sex, work-related symptoms, and atopic disease if any. The study was approved by the Medical Ethical Committee of our hospital and all participating subjects (or their parents if the subjects were younger than 18 years) gave their written informed consent.

Isolation of pollen

The isolation of pollen was carried out according to method described by Baer and Anderson (3). This method allows separating pollen grains with a purity > 90% from waste remains of plant and flowers. The purity was determined by observing five random samples of isolated pollens under an optical microscope. Three hundred particles were counted from each sample, and the proportion between *Sinapis* pollen grains and other contaminated particles (e.g. flower remains) was calculated.

Abbreviations: EAST, enzyme allergosorbent test; NCT, nasal challenge test; SPT, skin prick test.

Preparation of the extract

Deffated *S. alba* pollen was extracted by magnetic stirring in phosphate buffer, clarified by centrifugation, filtered through 0.45 µm pore diameter membranes and dialyzed by ultrafiltration with 5 kDa cutoff. The dialyzed extract was sterilized through a 0.22-µm pore diameter membrane and freeze-dried (4).

Quantitative skin tests

Skin prick tests were performed in accordance with the recommendations of the European Academy of Allergology and Clinical Immunology (5). Skin tests were carried out with *S. alba* pollen extract and a routine battery of aeroallergens of our area (*Lolium perenne*, *Olea europaea*, *Artemisia vulgaris*, *Chenopodium album*, *Salsola kali*, *Parietaria judaica*, *Plantago lanceolata*, *Dermatophagoides pteronyssinus*, *Alternaria alternata*, dog and cat danders) (Lab Bial, Bilbao, Spain).

Sinapis alba lyophilized pollen extract was diluted in phenol glycerol saline solution at concentration of 0.002, 0.02, 0.2, 2 and 20 mg/ml and prick test with standardized lancets were performed. Histamine chlorohydrate at 10 mg/ml and phenol-glycerol saline solution were used as positive and negative controls, respectively. After 15 min, cello tape was applied over the wheals and the areas transferred to a blank record sheet (areas expressed in square millimeters).

The mean wheal area produced by each allergen concentration was plotted as a function of the allergen concentration in a log-log system and linear regression was performed. The allergen concentration eliciting a wheal equal to that produced by histamine (10 mg/ml) was defined as one histamine-equivalent prick (HEP) unit (6). One HEP unit of *S. alba* pollen extract was found to be equivalent to 1.4 mg/ml.

Nasal challenge tests

Nasal challenge tests (NCT) were performed in 12 patients with work-related respiratory symptoms and positive skin tests to *S. alba* pollen, and in eight subjects from the control group. This study was carried out in 2004, outside of the *S. alba* pollination period, at 9 AM after adaptation to room temperature for 30 min, according to the method described by Gosepath et al. (7).

The response was evaluated by measuring nasal resistance with active anterior rhinomanometry and by scoring the clinical symptoms. Total symptom scores represented the sum of the scores for: sneezing (0–2 sneezes: 0 points; 3–5: 1 point; > 5: 2 points); rhinorrhea (moderate: 1 point; severe: 2 points); tearing, itching (eyes, throat): 1 point; conjunctivitis, cough, urticaria and/or dyspnea: 2 points. Positive clinical challenge was defined as > 3 points (7).

Active anterior rhinomanometry was performed according to the criteria of the Committee Report on Standardization of Rhinomanometry (8). A rhinospir 164 rhinomanometer (Sibelmed, Barcelona, Spain) programmed to perform nasal provocation test was used. Airflow and resistances were recorded in an X-Y mirror image.

After spraying 0.2 ml of the diluent, increasing concentrations of allergen (0.002, 0.02, 0.2, 2 and 20 mg/ml) were sprayed into the same nostril every 15 min until symptoms appeared and resistances doubled those induced by the diluent.

Specific IgE measurement

Specific IgE was measured by means of the enzyme allergosorbent test (EAST) against *S. alba* pollen. The solid phase was obtained by coupling the extract solution (10 mg/ml) to the 6-mm diameter cyanogen bromide-activated paper disks as described by Ceska and Lundqvist (9). EAST was performed in accordance with the manufacturer's instructions (Specific IgE EIA Kit; HYTEC.HY-COR Biomedical Ltd, Penicuik, UK).

Aerobiological sampling

A volumetric Hirst-type spore trap was used (10) for aerobiological sampling in the urban environment. This trap was placed 15–25 m above the ground level, ensuring free air circulation. We used a portable monitoring station (VPPS 1000 Lanzoni; Lanzoni s.r.l., Bologna, Italy) for the camp sampling, into an olive crop which contains high densities of *S. alba*. Sampling was carried out in a continuous way during the whole flowering period of the plant population during 2003.

Daily pollen counts were made using the methodology proposed by the Spanish Aerobiology Network (11) and the data are expressed as number of pollen grains per cubic meter of air (grains/m³).

Table 1. Clinical characteristics and specific anti-*Sinapis alba* IgE class of 12 patients sensitized to *S. alba* pollen

Case	Age (years)	Sex	Symptoms	Allergen sensitization	EAST- <i>Sinapis</i> (class)
1	24	M	BA, R	Lol, Art, Ole, Plan, Sal, cat, dog	3
2	21	M	BA, R	Lol, Ole, Art, Plan, Che, Sal, cat	3
3	27	M	BA, R	Lol, Ole, Plan, Che, Sal	2
4	27	M	BA, R	Lol, Ole, Art, Plan, Che, Sal	3
5	26	M	BA, R	Ole, Art, Par, Plan, Che, Sal	2
6	35	F	BA, R	Lol, Ole, Art, Par, Plan, Che, Sal	3
7	14	F	BA, R	Lol, Ole, Plan, Che, Sal, Art	4
8	24	M	BA, R	Lol, Ole, Par, Plan, Che, Sal	4
9	26	M	BA, R	Lol, Ole, Art, Plan	4
10	31	M	BA, R	Lol, Ole, Plan, Che, Sal	4
11	17	F	R	Lol, Ole, Par, Plan, Che, Sal	2
12	25	M	BA, R	Lol, Ole, Art, Par, Plan, Che, Sal	3

R, rhinitis; BA, bronchial asthma; Lol, *Lolium perenne*; Ole, *Olea europaea*; Art, *Artemisia vulgaris*; Che, *Chenopodium album*; Sal, *Salsola kali*; Par, *Parietaria judaica*; cat, cat dander; dog, dog dander; EAST, enzyme allergosorbent test.

Table 2. Nasal challenge test results in 12 patients with *Sinapis alba* sensitization

Case	Baseline resistance	Negative control resistance*	<i>S. alba</i> pollen extract concentration-positive NCT†	Resistance at positive NCT	Symptom score (points)
1	0.52	0.54	10 ⁰	1.29	5
2	0.77	0.44	10 ⁻⁴	0.88	4
3	0.20	0.25	10 ⁻³	0.57	4
4	0.30	0.27	10 ⁰	1.82	5
5	0.45	0.53	10 ⁻³	1.42	3
6	0.43	0.57	10 ⁻³	3.48	3
7	0.40	0.53	10 ⁰	0.95	5
8	0.77	0.61	10 ⁻³	1.35	3
9	0.30	0.21	10 ⁰	0.43	4
10	0.25	0.23	10 ⁰	0.52	4
11	0.34	0.42	10 ⁻²	1.07	6
12	0.39	0.46	10 ⁻¹	0.98	4

* Resistance after diluent (saline solution).

† 10⁰: 20 mg/ml; 10⁻¹: 2 mg/ml; 10⁻²: 0.2 mg/ml; 10⁻³: 0.02 mg/ml; 10⁻⁴: 0.002 mg/ml.

Resistance is expressed in Pa sec/cm³.

Results

The clinical features of the 12 patients are summarized in Table 1. They showed symptoms between January and March, exclusively in the work environment: five of them during weed control management and seven during harvesting.

They had positive results in skin prick tests (SPT) and NCT to *S. alba* pollen extract. The symptom scores and functional alterations during NCT are summarized in Table 2. Specific IgE to *S. alba* pollen was positive (>0.35 kU/ml) in all patient sera (Table 1).

The seasonal variation of the daily production of *S. alba* pollen during the study period is shown in Fig. 1. The total grain count of *S. alba* pollen during its main pollen season (31 days: between 15 February and 15 April) was 29.994 (with a mean value of 968 grains/m³) in the portable monitoring station placed in *S. alba* community, and 81 (with a mean value of 3 grains/m³) in the urban monitoring station.

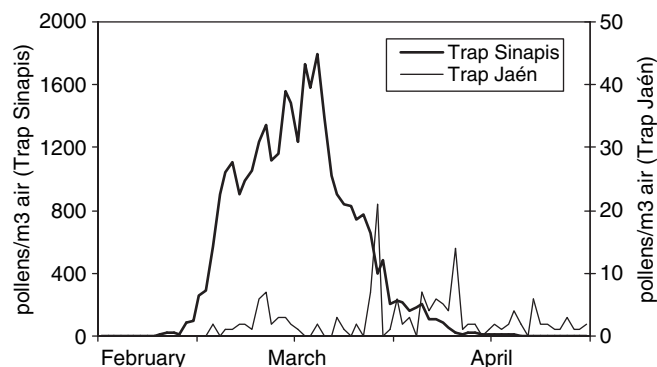


Figure 1. Daily concentration of airborne *Sinapis alba* (Trap *Sinapis*) pollen during the pollen season in contrast with airborne pollens from Brassicaceae species (Trap Jaén).

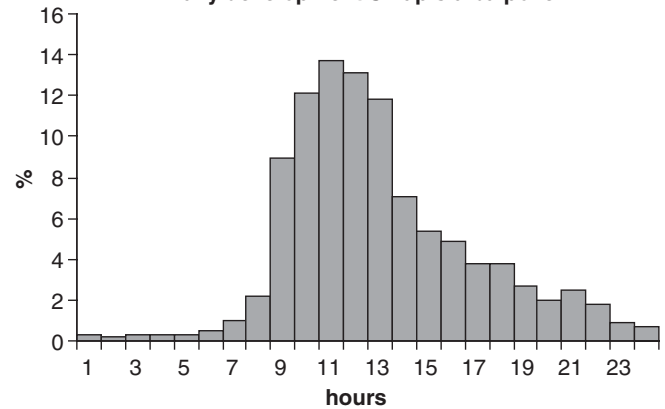
Daily development *Sinapis alba* pollen

Figure 2. Daily development of airborne pollen in *Sinapis alba* community. Percentage of *S. alba* pollen grain of each day hour against the total day *S. alba* pollen grain.

The highest amounts of pollen are released early in the morning (09:00–13:00), when floral anthesis occurs, whereas during night hours (23:00–07:00) the pollen emission to the atmosphere is suppressed (Fig. 2).

Discussion

We have demonstrated the existence of a new occupational allergen: *S. alba* pollen in olive farmers.

We demonstrate that exposure when working directly in olive orchard can be 300-fold higher than that observed in the general population. This broad difference probably reflects the aerobiological behavior of an entomophilic plant, with a limited pollen liberation rate into the atmosphere and in which the daily pattern of pollen emission may vary throughout the day (12). Other authors have also suggested the need of this aerobiological sampling of the patient's environment, with portable devices, for the detection and quantification of proximity aeroallergens, in some species of the Brassicaceae family (13).

After suspecting an occupational disease and precisely measuring the nature and timing of exposure in olive orchard workers, the diagnosis must be confirmed in each worker through inhalation challenges. Reports about the allergenicity of Brassicaceae pollen are limited, but they have applied this same diagnostic stepwise approach. *Diplotaxis erucoides* pollen has been identified as a relevant source of aeroallergens with capacity to induce IgE-mediated sensitization in agricultural workers, directly involved in viniculture, with clinical respiratory symptoms and occupational sensitization. These patients showed sensitization (SPT and specific IgE) to *D. erucoides* pollen and rhinoconjunctival symptoms after nasal inhalation challenges (14).

Thus, we have demonstrated the IgE-mediated nature of these reactions by means of the positive SPT and the

positive specific anti-*S. alba* IgE. Specific inhalation challenge with active anterior rhinomanometry confirmed that respiratory allergy was caused by *S. alba* pollen, eliciting an immediate clinical and physiologic response.

We conclude that *S. alba* pollen is a potential cause of IgE-mediated occupational respiratory disease.

Epidemiologic studies are necessary to assess the importance of this aeroallergen among exposed olive orchard workers and among the general population living nearby *S. alba*-growing areas.

References

1. Cano E, Ferro G, Torres JA, Cano-Ortiz A. Análisis de la biodiversidad florística de la provincia de Jaén (Andalucía-España). *Quad Bot Ambientale Appl* 2002;**13**:111–115.
2. Valdés B, Díez MJ, Fernández I. Atlas polínico de Andalucía Occidental. Sevilla: Instituto de desarrollo regional de la Universidad de Sevilla, 1987.
3. Baer H, Anderson M.Ch. Allergenic extracts. In: Middleton E, Reed CE, Ellis EF, Adkinson NF, editors. *Allergy: principles and practice*. St Louis: C.V. Mosby Company, 1992: 353–367.
4. García-González JJ, Bartolomé-Zavala B, Trigo-Pérez del Mar M, Barceló-Muñoz JM, Fernández-Meléndez S, Negro-Carrasco MA, et al. Pollinosis to *Ricinus communis* (castor bean): an aerobiological, clinical and immunochemical study. *Clin Exp Allergy* 1999;**29**:1265–1275.
5. Dreborg S. Skin test used in type I allergy testing (position paper). *Allergy* 1984;**39**:596–601.
6. Malling H-J. Quantitative skin prick testing. *Allergy* 1987;**42**:196–204.
7. Gosepath J, Amadee RG, Mann WJ. Nasal provocation testing as an international standard for evaluation of allergic and nonallergic rhinitis. *Laryngoscope* 2005;**115**:512–516.
8. Clement PAR. Committee report on standardization of rhinomanometry. *Rhinology* 1984;**22**:151–155.
9. Ceska M, Lundqvist U. A new and simple radioimmunoassay method for the determination of IgE. *Immunochemistry* 1972;**9**:102–105.
10. Hirst JM. An automatic, tric spore trap. *Ann Appl Biol* 1952;**39**:257–265.
11. Domínguez E, Galán C, Villamandos F, Infante F. Handling and evaluation of the data from the aerobiological sampling. *Monografías REA/EAN* 1991;**1**:1–18.
12. Ruíz-Valenzuela L, Díaz de la Guardia C, Quiralte J. Effect of density source in the dispersal of pollen: study in a population of *Sinapis alba* (Brassicaceae), an entomophylic plant. *Polen; XI Int Paly-nol Congr* 2004;**14**:345.
13. Fiorina A, Scordamaglia A, Guerra L, Passalacqua G. Aerobiological diagnosis of Brassicaceae-induced asthma. *Allergy* 2003;**58**:829–830.
14. García-Ortega P, Bartolomé B, Enrique E, Gaig P, Richart C. Allergy to *Diplotaxis erucoides* pollen: occupational sensitization and cross-reactivity with other common pollens. *Allergy* 2001;**56**: 679–683.