An occupational respiratory allergy due to Sinapis alba L. pollen in olive farmers

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<th>Journal:</th>
<th>Allergy</th>
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<td>Manuscript ID:</td>
<td>ALL-2006-00575</td>
</tr>
<tr>
<td>Manuscript type:</td>
<td>Original article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>22-Aug-2006</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Anguita, Juan Luis; Complejo Hospitalario de Jaén, Unidad de Alergología Palacios, Luis; Complejo Hospitalario de Jaén, Unidad de Alergología Ruiz, Luis; Facultad de Ciencias. Universidad de Jaén, Departamento de Botánica Bartolomé, Borja; Bial-Aristegui, R&amp;D Department López-Urbano, María José; Complejo Hospitalario de Jaén, Unidad de Alergología Saenz de San Pedro, Blanca; Complejo Hospitalario de Jaén, Unidad de Alergología Cano, Eusebio; Facultad de Ciencias. Universidad de Jaén, Departamento de Botánica Quiralte, Joaquín; Complejo Hospitalario de Jaén, Unidad de Alergología</td>
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TITLE: An occupational respiratory allergy due to *Sinapis alba* L. pollen in olive farmers.

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SUMMARY

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

Methods: The twelve patients were olive orchard workers and they had a history of rhinitis and/or bronchial asthma that occurred during control weed management and/or harvest, from January to March. They were skin tested (SPT) with *S. alba* pollen extract, mustard seed extract and a standard battery of aeroallergens. *S. alba* pollen extract was prepared to perform quantitative skin tests, enzyme allergosorbent test (EAST), nasal challenge tests (NCT), and EAST inhibition assay. 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 *Sinapis alba* pollen extract, and they showed a positive NCT response. EAST-inhibition assay showed a 60 - 75% inhibition value with other pollen extracts (*Artemisia vulgaris, Parietaria judaica, Lolium perenne, Olea europaea, Plantago lanceolata*). In the urban aerobiologic monitoring station the amount of *S. alba* pollen only exceptionally reaches peaks of 21 grains / m³, whereas in the work environment peaks of 1801 grains / m³ were detected between 15 February to 7 April.

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

**KEY WORDS:** bronchial asthma, occupational respiratory allergy, olive farmers, rhinitis, *Sinapis alba* pollen.

**Abbreviations used:**
EAST, Enzimo AllergoSorbent Test
NCT, Nasal Challenge Test
SPT, Skin Prick Test
Introduction

The olive farming is a key element of the agriculture in Andalucía, a Spanish region, and the southeast region of the European Union (EU). The olive oil produced in this area represents more than 80 percent of the Spanish production of olive oil, around 40 % of EU production and more than 30 % of the world production. The sector involves about 500 thousands producers in Jaén and it is an important economic activity and source of employment, providing seasonal employment in winter and significant off-farm activity in the associated milling and processing industries (1).

Olive trees require the same high level of management as other commercial tree crops. Weed management is an important part of the overall orchard management system, because it enhances the development of newly planted trees, improves the growth and yield of established trees, avoid pests and ameliorate certain cultural practices and the harvest.

There are a lot of different species of annual weeds which infest the olive orchard (2). These plants have a quick growth, an abundant flowering, and they show a high capacity of invading olive cultivars in suitable environmental conditions, as it happens with some species of Gramineae, Chenopodiaceae or Asteraceae families(3). Species of Brassicaceae family are also frequently found among these colonizer plants (Sinapis ssp., Diplotaxis ssp, Eruca ssp., Sisymbrium ssp., Moricandia ssp. etc.) (4). In olive orchard of Jaén, Sinapis alba (white
mustard) a 20-100 cm weed with an intensely yellow flowers, is one of the most frequent colonizer plant in the olive grove (5).

Weeds control and harvesting are two critical activities in olive crop care. Weeds are controlled either chemically or mechanically in a 2 to 5 foot wide strip in the tree and often both management systems are combined. In the Mediterranean area the harvesting methods are hand-made and farmers usually work at the base of mature trees, collecting and first processing the olive fruits. It is important to note that both manoeuvres imply a narrow contact with olive orchad, at the time in which Sinapis alba is flowering (late winter to early spring). Like other Brassicaceae species, the pollen of this plant is prolate or subspheroidal, ranging from small to medium size, trizonocolporate, isopolar, radioisometric with a reticulate surface (6).

The aim of this study was to investigate the cause of the weed management and/or harvesting-related respiratory symptoms showed by 12 olive orchard workers. We involved Sinapis alba pollen as the cause of this respiratory disease through specific nasal challenge, and we also established its specific aerobiological patterns. Finally, we demonstrated the presence of cross-reactivity between this pollen and those from other plants.
Patients and methods

Patients

Twelve patients (9 males and 3 females) with ages ranged from 14 to 35 years (mean age 24.7 ± 5.6 years) were included in the study. Patients fulfilled the following criteria: 1) they were olive orchard workers; and 2) they had a history of rhinitis and/or bronchial asthma that occurred during control weed management and/or harvest during late winter (January to March).

The clinical data of the patients: age, sex, work-related symptoms and atopic disease if any. Subjects were diagnosed as being atopic if they had rhinitis and/or bronchial asthma, with 1) skin prick wheal of 3 mm greater than negative control and 2) specific IgE values > 0.7 kU/L measured by Pharmacia ImmunoCap System (Pharmacia Diagnostics, Sweden) to one or more of house dust mites, fungi, animal danders or pollen extracts

Written informed consent from the patients (or their parents if they younger than 18 years old) was necessary for participation in the study, and the protocol was approved by the Investigation and Ethics Committees of our Hospital.

Isolation of pollen

The suitable selection of the vegetable material is essential in order to assure the maturity of pollen grain with the purpose that it maintain a good allergenic activity. The isolation of pollen was carried out according to method described by Baer H. et al (7). We extracted directly pollen of the mature flowers before opening and we allowed them to dry on a sieve with diameter of mesh of 25
μm. When the inflorescences are dry they are crushed smoothly and sifted, where
the pollen falls down on clean paper. This method allows to separate the grains of
pollen, with a greater purity of the 90%, of waste sing like remains of plant and
flowers. For their conservation the grains of pollen are dried in stove at 35-40 °C
maintaining less humidity than 7%. Finally the pollen grains are stored in fridge.

**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 dialysed by ultrafiltration with 5 kDa cutoff. The dialyzed extract
was sterilized through a 0.22 μm pore diameter membrane and freeze-dried (8).

**Quantitative skin test**

Skin prick tests were performed in all patients according to the
recommendations of the European Academy of Allergology and Clinical
Immunology (9). Skin tests were carried out with *S. alba* pollen extract, mustard
seed extract (Lab Bial, Bilbao, Spain) and with the routine battery of aeroallergens
of our area. This battery included *Lolium perenne*, *Olea europaea*, *Artemisia
vulgaris*, *Chenopodium album*, *Salsola kali*, *Parietaria judaica* and *Plantago
lanceolata*, *Dermatophagoides pteronyssinus*, *Alternaria alternata*, dog and cat
danders (Lab Bial, Bilbao, Spain)

*Sinapis alba* lyophillized 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 Morrow-Brown lancets were performed. Histamine clorhidrate at 10
mg/mL and phenol glycerol saline solution were used as positive and negative controls, respectively. After 15 minutes, sellotape was applied over the wheals obtained, and the areas transferred to a blank record sheet, areas were 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 (10). One HEP unit of *S. alba* pollen extract was found to be equivalent to 1.4 mg/mL.

**Nasal challenge test**

Nasal challenge test (NCT) was performed in 12 patients with work-related respiratory symptoms and positive skin test response to *S. alba* pollen, and in 8 subjects from the control group (5 atopic patients and 3 otherwise normal subjects without *S. alba* sensitization). This study was carried out between 2003 and 2004 outside the *S alba* pollination period and the patients had not taken any medication during the previous month. Prior to study, functional respiratory tests were made following the American Thoracic Society guidelines (11), and nasal exploration ruled out any obstruction of the nostrils.

Active anterior rhinomanometry was performed according to the criteria of the Commitee Report on Standardization of Rhinomanometry (12). A rhinospir 164 rhinomanometer (Sibelmed, Spain) programmed to perform nasal provocation test was used. Airflow and resistances were recorded in a X-Y mirror image. After
spraying 0.2 mL of diluent, increasing concentrations of allergen (0.002, 0.02, 0.2, 2 and 20 mg/mL) were sprayed into the same nostril every 15 minutes until symptoms appeared and resistances doubled those induced by the diluent.

**Specific IgE measurement (EAST)**

Specific IgE were measured by means of EAST technique (Enzyme AllergoSorbent Test) against different types of pollens (*Sinapis alba, Chenopodium alba, Parietaria judaica, Olea europaea, Plantago lanceolata, Artemisia vulgaris, Salsola kali, Lolium perenne*). Solid-phase was obtained by coupling the extracts solution (10 mg/mL) to the 6-mm diameter CNB-activated paper discs as described by Ceska and Lundqvist (13). EAST was performed in accordance with the manufacturer’s instructions (Specific IgE EIA kit. HYTEC.HYCOR Biomedical Ltd. UK)

**EAST inhibition tests**

EAST-inhibition was performed according to methods reported by Yman et al (14). A serum pool from all the patients and a serial dilution (0.001, 0.01, 0.1, 1, 10 mg/mL) of the inhibitor pollen extract were used to carried out the assay. The Ag50 value is defined as the concentration (mg/mL) of the extract which produced a 50% inhibition in the assay.

**Aerobiological sampling**

Aerobiological sampling were collected in two different sites: an urban environment and into an olive crop which contain high densities of *S. alba*. A volumetric Hirst-type spore trap was used (15) for the aerobiological sampling in the urban environment; this trap was located on the roof of the Departament of
Botany in the University of Jaén, at 15-25 m above the ground level, ensuring free air circulation. We used a portable monitoring station (VPPS 1000 Lanzoni) near a great population of *Sinapis alba* (Brassicaceae) for the camp sampling. Sampling was carried out in a continuous way during the whole flowering period of the plant population during 2003. This allowed to study the evolution of the *S. alba* pollen concentration in relation to the open flower densities. Observed data were compared to those reported about *Brassicaceae* species by the aerobiological station located in Jaén (Burkard spore-trap).

Daily pollen counts were made using the methodology proposed by REA (Spanish Aerobiology Network) (16) and the data are expressed as a number of pollen grains per cubic metre of air (grains/m\(^3\)).
Results

Patients

The clinical features of the 12 patients were summarized in Table 1. Out of them, 11 had rhinitis and bronchial asthma and one only rhinitis. They had positive results in skin prick test and NCT to Sinapis alba pollen extract. They showed symptoms between January to March in the work environment: 5 of them during weed control management and 7 during harvesting.

Nasal challenge tests

The clinical characteristics and functional alterations during NCT were summarized in Table 2. Five patients showed a positive response at 20 mg/mL of S. alba pollen extract and the remaining 7 patients showed a nasal response at values below 2 mg/mL. All patients had clinical symptoms (mainly blockage, rhinorrea and pruritus, alone or in combination). No symptoms and/or resistances alterations were observed after NCT with S. alba in 8 control patients.

Specific IgE measurements

Specific IgE against pollens extracts was measure by means of EAST method. Specific IgE to S. alba pollen was positive (> 0.35 kU/mL) in all patient’s sera: 4 of them (33%) were class 4, five (42%) were class 3 and three of them (25%) were class 2 (see Table 1). Specific IgE value higher than 3.5 kU/L (class 3 or 4) was obtained in ten sera (83%) against pollens from Chenopodium album, Olea europaea, Salsola kali, Lolium perenne, in eight sera (67%) against Plantago lanceolata pollen, in six sera (50%) against Artemisia vulgaris pollen and in 5 cases (41 %) against Parietaria judaica.
Cross-reactivity studies

Cross-reactivity studies were carried out with EAST-inhibition assays (figure 1). When *S. alba* pollen extract was used as inhibitor, an $Ag_{50} = 5 \mu g/mL$ and 100% inhibition at 1 mg/mL was obtained, whereas when other pollen extracts were used the following values of $Ag_{50}$ and inhibition percentages at 1 mg/mL were obtained: *Plantago lanceolata* $Ag_{50} = 15 \mu g/mL$ and 75% inhibition; *Olea europaea* $Ag_{50} = 24 \mu g/mL$ and 74% inhibition; *Lolium perenne* $Ag_{50} = 96 \mu g/mL$ and 68% inhibition; *Parietaria judaica* $Ag_{50} = 140 \mu g/mL$ and 63% inhibition; *Artemisia vulgaris* $Ag_{50} = 200 \mu g/mL$ and 60% inhibition.

Aerobiological studies results

The seasonal variation of the daily production of white mustard pollen during the studied period is shown in Figure 2. The total grain count of *S. alba* pollen during its main pollen season of *S. alba* (31 days: between 15 february to 15 april) was 29.994 (with a mean value of 968 grains/m$^3$) in portable monitoring station placed in *S alba* community, and 81 (with a mean value of 3 grains/m$^3$) in the urban monitoring station.

The daily evolution of pollen concentration is summarized in figure 3. The highest amounts of pollen are liberated early in the morning (9 - 13 hours), when floral anthesis occurs, whereas during the night hours (23 - 7 hours) the pollen emission to the atmosphere is suppressed.
Discussion

We have demonstrated the existence of a new occupational allergen: Sinapis alba pollen in olive farmers. If this allergen represent a disease-associated factor for experiencing respiratory allergy, we would find both evidences of higher level of exposure to this pollen in the environment in which these patients worked and also a clinical response after controlled allergen inhalation challenge, in whom these symptoms occurred, and this is what we have found.

We have been able to demonstrate that exposure when working directly in olive orchard can be 300-fold higher that observed in general population. This broad difference between both measurements probably reflects the aerobiological behaviour of an entomophilic plant (17), with a limited pollen liberation rate into atmosphere and in which daily pattern of pollen emission may vary throughout the day (18). Other authors has also suggested the need of this aerobiological sampling, with portable devices, of the patients’s environment, for the detection and quantification of allergen burden in some species of the Brassicaceae family (19). Thus, the sampling of a restricted area can evidence the proximity aeroallergens which remain confined near their source, but which can provoke severe respiratory diseases in sensitive patients who were in close contact with these plant communities.

If we have suspected an occupational disease and we have precisely measured the nature and timing of exposure in our olive orchard workers, the next must be confirming a diagnosis in each individual workers, 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 (20), directly involved in viniculture, with clinical respiratory symptoms and occupational sensitization. These patients showed sensitization (SPT and specific IgE) to *Diplotaxis erucoides* pollen and rhinoconjunctival symptoms after nasal inhalation challenges.

Thus, we have demonstrated the IgE-mediated nature of these reactions by means of the positive SPT and the positive specific anti-*Sinapis alba* IgE. Specific inhalation challenge with active anterior rhinomanometry confirmed that respiratory allergy was due to *S. alba* pollen, eliciting an immediate clinical and physiologic response.

*Sinapis alba*, known as white mustard, is one of the species used to obtain the mustard seeds. Mustard allergy is a disorder that can induce severe hypersensitivity reactions (21, 22, 23). A major allergen of white mustard, Sin a 1, has been characterized as a seed storage protein, belonging to the 2S albumin family, with a molecular weight (MW) of 14 Kda (24, 25, 26). In addition, an 11 S globulin storage protein (Sin a 2), with a molecular mass of 51 Kda, has been isolated and identified as a novel major allergen of mustard seeds (27).

However, none of our patients referred allergy to mustard seed (data not shown), in spite of they showed challenge-proven respiratory allergy to *S. alba* pollen. Moreover, all patients have also other concomitant pollnosis (see Table 1).
The cross-reactivity studies, carried out with EAST-inhibition assays, showed a 60-75% inhibition value with heterologus extract used as free phase (Artemisia vulgaris, Parietaria judaica, Lolium perenne, Olea europaea, Plantago lanceolata). These results prove different grades of allergenic similitude between Sinapis alba pollen and the others prevalent pollens in our area (Figure 1).

We concluded that S. alba pollen is a potential cause of IgE mediated occupational respiratory disease. Epidemiological studies are necessary to assess the importance of this aeroallergen among exposed olive orchard workers and among the general population nearby the Sinapis alba growing areas.
References


Legend of figure 1: EAST - inhibition results. *S. alba* pollen extract used as solid phase and different pollen extracts as inhibitor phase.

Legend of figure 2: Daily concentration of airborne *Sinapis alba* (Trap Sinapis) pollen during the pollen season in contrast with airborne pollens from Brassicaceae species (Trap Jaén).

Legend of figure 3: 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.
<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Symptoms*</th>
<th>Allergen sensitization</th>
<th>EAST-sinapis (class)</th>
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<td>21</td>
<td>M</td>
<td>BA, R</td>
<td>Lol, Ole, Art, Plan, Che, Sal, Cat</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>M</td>
<td>BA, R</td>
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<td>2</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>M</td>
<td>BA, R</td>
<td>Lol, Ole, Art, Plan, Che, Sal</td>
<td>3</td>
</tr>
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<td>5</td>
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<td>M</td>
<td>BA, R</td>
<td>Ole, Art, Par, Plan, Che, Sal</td>
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</tr>
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</tbody>
</table>

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

* R: rhinitis,  BA: bronchial asthma.


EAST, Enzyme AllergoSorbent Test
Table 2. Nasal challenge test results in 12 patients with *Sinapis alba* sensitization.

<table>
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<tr>
<th>Case</th>
<th>Baseline Resistance</th>
<th>Negative control Resistance*</th>
<th>S. <em>alba</em> pollen extract concentration positive NCT*</th>
<th>Resistance at positive NCT</th>
<th>NCT Symptoms</th>
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<td>1</td>
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<td>0.54</td>
<td>$10^0$</td>
<td>1.29</td>
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<td>0.77</td>
<td>0.44</td>
<td>$10^{-4}$</td>
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</tr>
<tr>
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<td>0.20</td>
<td>0.25</td>
<td>$10^{-3}$</td>
<td>0.57</td>
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<tr>
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<td>0.27</td>
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<td>0.45</td>
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<tr>
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<td>0.43</td>
<td>0.57</td>
<td>$10^{-3}$</td>
<td>3.48</td>
<td>Rhinorrea, conjunctivitis,</td>
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<td>7</td>
<td>0.40</td>
<td>0.53</td>
<td>$10^0$</td>
<td>0.95</td>
<td>Nasal pruritus and blockage, rhinorrea, bilateral conjunctivitis</td>
</tr>
<tr>
<td>8</td>
<td>0.77</td>
<td>0.61</td>
<td>$10^{-3}$</td>
<td>1.35</td>
<td>Nasal pruritus and blockage</td>
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<tr>
<td>9</td>
<td>0.30</td>
<td>0.21</td>
<td>$10^0$</td>
<td>0.43</td>
<td>Sneezes, nasal pruritus</td>
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<tr>
<td>10</td>
<td>0.25</td>
<td>0.23</td>
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<td>11</td>
<td>0.34</td>
<td>0.42</td>
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<td>Sneezes, nasal blockage, bilateral conjunctivitis</td>
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<tr>
<td>12</td>
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<td>0.46</td>
<td>$10^{-1}$</td>
<td>0.98</td>
<td>Sneezes, nasal blockage, rhinorrea</td>
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* Negative control Resistance: Resistance after diluent (saline solution)

**S. *alba* pollen extract concentration positive NCT: $10^0$: 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.

Resistances are expressed in pascale.
Figure 1.
Figure 2.
Figure 3.