
Vine pollen allergy in areas with a high density of vineyards

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Background: In Castilla-La Mancha, Spain, the area with the highest density of vineyards in the world, 2 cases of *Vitis vinifera* pollen allergy have been previously reported.

Objective: To determine the clinical relevance and biochemical characteristics of vine pollen in the Spanish province of Ciudad Real.

Methods: We designed a prospective study of patients treated in the allergy units from Puertollano and Ciudad Real for respiratory symptoms of 4 months' duration in the year 2000. Skin prick tests with a standard aeroallergen battery and *V. vinifera* pollen extract were performed on all patients. We also performed conjunctival and bronchial provocation tests and serum specific IgE and sodium dodecyl sulfate–polyacrylamide gel electrophoresis immunoblotting on the patients who tested positive for *V. vinifera* pollen.

Results: We included 200 patients, 98 sensitized to any pollen and 9 to *V. vinifera* pollen. We found 8 of 9 positive conjunctival provocation test results and a positive bronchial provocation result to vine pollen in a vine grower. Serum specific IgE against *V. vinifera* pollen was detected in 9 of 9 patients. Immunoblotting with *V. vinifera* pollen extract showed IgE-binding bands at 45 and 67 kDa.

Conclusions: Vine pollen could be the cause of pollinosis in exposed patients living in areas with a high density of vineyards.

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INTRODUCTION

The vine (*Vitis vinifera*) is a plant adapted to a vast range of climates, from the cold areas of Russia to the desert regions of California and from the temperate Mediterranean regions to the continental regions of Central Europe. The arid ground and extreme continental climate in Ciudad Real (Castilla-La Mancha, Spain) make this an ideal area for grape farming, with a total of 235,015 hectares dedicated to this crop (41.2% of arable farms), constituting the area with the highest concentration of vineyards in the world. Additionally, social involvement in grape farming and harvesting is high, with the participation of 20% to 30% of the population (70,000 families) in some areas.¹

In 1999, we diagnosed a female patient as having rhinoconjunctivitis and seasonal asthma, with symptoms that worsened after spending time in areas near vineyards. The allergy study, which used the skin prick test (SPT), specific IgE immunoblotting, and bronchial provocation test (BPT) with vine pollen, confirmed the role of this allergen in the patient's hay fever.² We subsequently described another female patient

with seasonal rhinoconjunctivitis due to allergy to vine pollen; this patient presented with episodes of urticaria after eating grapes.³ The immunochemical analysis demonstrated that the simultaneous allergy to vine pollen and grapes was related to the presence of common allergenic structures in the grape and on vine pollen. These first 2 cases of allergy to vine pollen led us to design a prospective study to determine the clinical relevance of allergy to this pollen in the Spanish province of Ciudad Real and to study the biochemical characteristics of its allergens.

METHODS

Patients

Patients with suspected respiratory allergy (rhinoconjunctivitis and/or asthma) attending the allergology outpatient clinics in Ciudad Real and Puertollano in January through April 2000 were included in the study.

Preparation of *V. vinifera* Pollen Extract

V. vinifera pollen was collected in the Ciudad Real province in the spring of 1999. The purity of the pollen collected was studied by microscopic particle count in 95% ethanol; 20 fields were examined at 400× magnification, with a total count of 740 pollen grains. The pollens were identified by electronic microscopy. Defatted pollen from these specimens was extracted by magnetic stirring (24 hours at 4°C) in 0.1-mol/L phosphate buffer (pH 8.0) at 15% (wt/vol). The extract was clarified by centrifugation at 5,600g for 30 minutes, filtered through a 0.45-μm pore diameter membrane, and dialyzed by ultracentrifugation (Pellicon System; Milli-

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pore Corp, Milford, Massachusetts) with a 5,000-day cutoff point. The dialyzed extract was sterilized by filtration through a 0.22- μ m pore diameter membrane and freeze-dried.

Pollen collection revealed 90% purity, with 1% contaminants per mixed pollens and 9% of plant particles other than pollen. Pollen extraction yielded 4 g of lyophilized powder derived from 100 g of pollen. Protein content was 10% (10 g of protein per 100 g of lyophilized powder), using the Bradford method.⁴

Skin Prick Test

The SPT was performed according to the method described by the Subcommittee on Skin Tests of the European Academy of Allergology and Clinical Immunology⁵ (using standardized lancets from Dome Hollister, Leverkusen, Germany), with a standard aeroallergen battery that included pollen from *Lolium perenne*, *Olea europaea*, *Salsola kali*, and *Artemisia vulgaris*, as well as *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus*, and cat and dog dander (BIAL-Arístegui Laboratories, Bilbao, Spain). An SPT was also performed with a *V. vinifera* pollen extract (5 mg/mL, BIAL-Arístegui Laboratories). Histamine chlorhydrate, 10 mg/mL, and phenolated glycerol saline solution were used as positive and negative controls, respectively. Ten control subjects (5 nonatopic subjects and 5 patients allergic to pollen) were also tested with *V. vinifera* pollen at a concentration of 5 mg/mL.

Conjunctival Provocation Test

The conjunctival provocation test was performed in patients with positive SPT results to vine pollen, in accordance with the method described by Jiménez et al.⁶ Vine pollen extract was used at concentrations of 0.1, 1, 2, and 5 mg/mL.

The test started with the administration of 1 drop of diluent (phosphate-buffered saline) into the conjunctival sac followed by monitoring of ocular symptoms for 20 minutes. If no reaction was observed, the lowest test concentration (0.1 mg/mL) was applied to the other eye. This operation was repeated using progressively increasing concentrations (0.1, 1, 2, and 5 mg/mL) until the highest concentration was reached, unless a positive response was detected. The conjunctival test response was considered positive with any of the following changes: chemosis, erythema that involved more than 50% of the bulbar conjunctival area, or erythema that involved less than 50% of the bulbar conjunctival area with itching or epiphora.⁷ Specificity controls were performed using the same schedule in 5 healthy subjects and in 5 patients with pollen allergy.

Bronchial Provocation Test

A specific BPT was performed with vine pollen extract in patients with asthma symptoms and positive SPT results to vine pollen, basically according to the method of Chai et al⁸ but with certain modifications. The patient was tested when asymptomatic, with a forced expiratory volume in 1 second (FEV₁) of greater than 80% of predicted normal value, and

when no medication had been required for several days. A control challenge with phosphate-buffered saline was performed before antigen challenge. The patient was skin tested at concentrations of 1, 2, and 5 mg/mL of vine pollen extract to determine a safe starting dose for the provocation test; this concentration was 0.1 mg/mL of extract (10-fold lower than the one that produced a 3 $\times$ 3-mm wheal). The patient inhaled the aerosolized allergen in progressively increasing concentrations (0.1, 1, and 5 mg/mL) for 2 minutes at tidal volume. Dilutions were administered with a Hudson 1720 nebulizer with input and output rates of 7 L/min and 0.28 to 0.30 mL/min, respectively. FEV₁ and peak expiratory flow rate (PEFR) were measured after each inhalation and also at 5, 10, 15, 30, and 60 minutes. To evaluate the bronchial late response, the patient monitored PEFR values and performed spirometry recordings using a portable Mini-Wright type peak flowmeter at home for a further 24 hours. A positive test response was defined as a decrease in FEV₁ of 20% or in PEFR of 25% from the postdiluent value during early or late determinations. Two atopic patients with mild asthma were also challenged with the same extract as the control subjects. Additionally, another 2 patients allergic to pollens common in the Ciudad Real area were challenged.

Specific IgE Determination

Serum specific IgE to vine pollen extract was determined by the enzyme allergosorbent test (EAST) technique. Solid-phase antigen was obtained by coupling the extract solution (10 mg/mL of extract solution; 40 μ g of protein per disk) to the 6-mm-diameter cyanogen bromide-activated paper disks, following the method described by Ceska and Lundqvist.⁹ EAST was performed according to the method of Wide et al,¹⁰ and results were expressed in accordance with the manufacturer's instructions (Specific IgE EIA kit; HYTEC HYCOR Biomedical Inc, Kassel, Germany), and values equal to or higher than 0.35 kU/L were considered positive. A pool of sera from nonallergic subjects was used as the negative control.

SDS-PAGE Immunoblotting

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method described by Laemmli.¹¹ Polyacrylamide concentrations of 12.5% and 4% were used for separating and stacking gels, respectively. Protein dissolved in 0.125M Tris hydrochloride, pH 6.8, was dissociated with 0.1% SDS and 5% β -mercaptoethanol by treatment at 100°C for 5 minutes; 20 μ g of protein was applied per lane.

After electrophoresis, the separated protein bands were electrophoretically transferred to polyvinylidene difluoride membranes (Immobilon-P; Millipore, Bedford, Massachusetts), essentially according to Towbin et al.¹² After blocking (9% defatted dry milk in Tris-buffered saline for 1 hour at 37°C), membranes were incubated overnight at 4°C with patient serum, incubated with antihuman IgE-horseradish peroxidase conjugate (1/5,000 dilution) from Southern Bio-

tech (Birmingham, Alabama) and detected using the chemiluminescence method as recommended by the manufacturer (ECL-Plus; Amersham Pharmacia Biotech, Uppsala, Sweden).

RESULTS

Vine pollen is a small (18 to 25 μ m) tricolporate, suboblate, or oblate pollen. *V vinifera* pollen is similar to *Quercus ilex* pollen on light microscopy and also pollinates in the same period (May-June). They differ in the size of the pores, which are larger in *V vinifera* pollen (Figs 1 and 2).

A total of 200 patients (96 males and 104 females), with ages ranging from 14 to 42 years (mean, 25.37 years), were included in the study. The patients presented with a previous history of rhinitis (55.8%), asthma (13.4%), or rhinitis plus asthma (30.8%).

We found 98 patients with seasonal respiratory symptoms and positive SPT results to pollens, of whom 9 had a positive SPT response to *V vinifera* pollen. The demographic characteristics, clinical symptoms, other sensitizations, and serum specific IgE value against vine pollen extract of the 9 patients studied are summarized in Table 1.

The results of the conjunctival provocation tests performed with the extract of *V vinifera* pollen were positive in all 9 patients except for patient 5. The BPT result was positive in all patients tested: patients 1, 3, and 7 (patient 7 was a farmer) at a concentration of 5 mg/mL, with maximum decreases of 21%, 20%, and 22%, respectively, in the FEV₁ and of 26%, 29%, and 31%, respectively, in the PEFR. No delayed reaction was detected in these 3 patients on peak flow meter testing during the 24 hours after the BPT.

SPT results to *V vinifera* pollen varied from 9 to 48 mm². Serum specific IgE levels to *V vinifera* pollen extract (EAST) ranged from 0.42 to 10.11 kU/L. EAST class 3 was detected in 1 serum sample, 5 serum samples showed EAST class 2, and 3 serum samples showed EAST class 1; the value obtained with a pool from nonatopic subjects was less than 0.35 kU/L (class 0). SDS-PAGE after Coomassie stain showed *V*

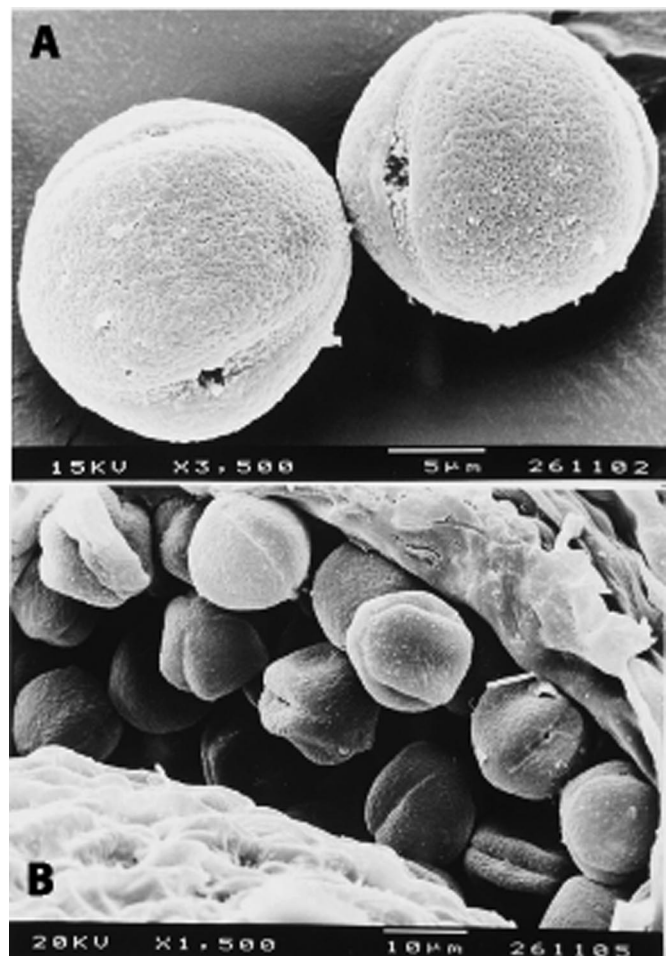


Figure 2. Scanning microscopy image of *Vitis vinifera* pollen (A) and pollen sac (B). Microphotography donated by M. Jerez (Real Jardín Botánico, Madrid).

vinifera pollen protein levels that ranged from 27 to 70 kDa. Immunoblotting with *V vinifera* pollen extract showed IgE-binding bands at 45 and 67 kDa with the serum from 3 subjects (Fig 3). None of the control subjects had a positive response to the SPTs, conjunctival provocation tests, or BPTs.

DISCUSSION

Vine (*V vinifera*) pollen is airborne, and its presence can be detected in the atmosphere in Ciudad Real during the months of May and June, reaching peak concentrations of 15 to 20 grains per cubic meter of air.² The results of this prospective study show that, in our region, this pollen has a moderate clinical relevance from an allergic point of view, particularly affecting those subjects who are exposed to it during their working hours and those who perform leisure activities close to vine fields.

During a period of 4 months, we performed SPTs to vine pollen in patients who attended our outpatient clinic with

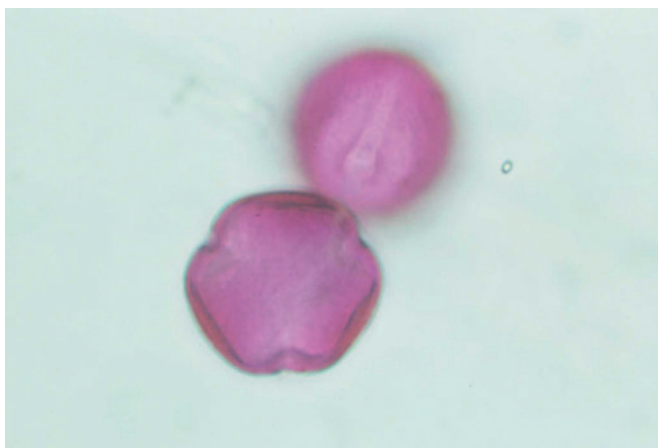


Figure 1. *Vitis vinifera* pollen viewed by light microscopy.

Table 1. Characteristics of 9 Patients Sensitized to *Vitis vinifera* Pollen

Patient No./sex/age, y	Profession	Other sensitizations	Symptoms	EAST result, kU/L	EAST class	SPT result, mm ²
1/F/16	Student	Pollen from grass, olive, <i>Plantago lanceolata</i> , and dog dander	R, C, A	1.5	2	24
2/M/20	Student	Pollen from grass, olive, <i>Salsola kali</i>	R, C	0.42	1	20
3/F/22	Waitress	Pollen from grass, olive	R, C, A	1.44	2	16
4/F/16	Student	Pollen from olive, <i>Salsola kali</i>	R, C	0.54	1	9
5/F/34	Teacher	Pollen from grass and cat dander	R, C	0.45	1	12
6/M/29	Electrician	Pollen from olive, <i>Salsola kali</i> , <i>Cupressus arizonica</i> , and <i>Alternaria alternata</i>	R, C	3.17	2	25
7/F/24	Farmer	Pollen from grass, olive and <i>Dermatophagoides pteronyssinus</i>	R, C, A	10.11	3	48
8/M/38	Policeman	Pollen from grass, <i>Salsola kali</i>	R, C	0.85	2	20
9/M/19	Student	Pollen from grass, olive, and cat dander	R, C	1.45	2	36

Abbreviations: A, asthma; C, conjunctivitis; EAST, enzyme allergosorbent test; R, rhinitis; SPT, skin prick test.

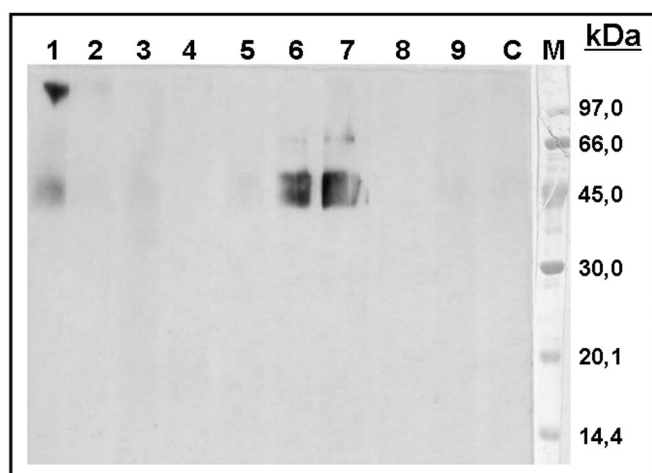


Figure 3. Immunoblot analysis with vine pollen extract. Lanes 1 to 9, patients' sera; lane C, control serum (pool from nonatopic subjects' serum samples); and lane M, molecular mass marker.

suspected respiratory allergy, finding sensitization to this pollen in 9 of the 200 patients seen (4.5% of the total, 9% of the 98 patients with pollen allergy). Furthermore, measurement of serum specific IgE and specific bronchial and conjunctival provocation tests confirmed the clinical relevance of the sensitization to this pollen. Five of the 9 sensitized patients (patients 1, 2, 5, 6, and 9) referred a worsening of their respiratory symptoms during the months of May and June (pollination period of vine pollen), when they passed close to areas with vineyards, and 1 patient, a farmer (patient 7), also presented with asthma in this period when working in the vineyards. These findings demonstrate the role of vine pollen in the respiratory symptoms of our patients.

Grape allergy had only been described in case reports¹³ until Pastorello et al¹⁴ analyzed 14 patients allergic to grape or wine and identified endochitinase 4 and a lipid transfer protein as major allergens in grape-allergic patients who did not have vine pollen allergy (some patients presented with allergy

to other pollens: grasses, *Parietaria*, *Artemisia*, birch). Similarly, Schad et al¹⁵ described a patient with severe anaphylactic reactions after consuming wine or grapes. They described an IgE-binding protein of 15 kDa, identified as grape lipid transfer protein (Vit v 1). However, in our series, the serum from 3 patients revealed IgE binding bands at 45 to 50 kDa, similar molecular masses to the bands detected when the serum from a patient allergic to grape and vine pollen was used.³ The other 6 serum samples did not reveal any IgE-binding bands, probably because of their low serum specific IgE concentration against *V. vinifera* pollen and the SDS protein denaturalizing effect. These data suggest that vine pollen allergens differ to some degree from those of the grape. Furthermore, because some vine pollen allergens have been shown to be cross-reactive with proteins from other botanically unrelated pollens, such as *Olea europaea*, *Lolium perenne*, and *Salsola kali*,³ patients sensitized to these pollens could experience an exacerbation of their symptoms when exposed to vine pollen in grape-farming areas.

Pollen allergy occasionally shows a local profile because it is not always due to the most allergenic plants with the widest distribution in each region; rather, the climatic conditions and the vegetation of the region can favor the atmospheric presence of other pollens able to cause symptoms in exposed individuals. This is the case for allergy to the Australian pine (*Casuarina*)¹⁶ and *Mercurialis annua*¹⁷ pollen, the clinical relevance of which can pass undetected or for which patient symptoms can be masked as they coincide with other, more allergenic pollens present at higher concentrations. Similarly, vine pollination in our region must not be underestimated because it coincides with grass and olive and could act as a hidden allergen in some sensitized patients.¹⁸ In conclusion, in areas with a high density of vineyards, vine pollen can reach midrange air concentrations and could be the cause of hay fever in those individuals with the highest levels of exposure.

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