

Rhinoconjunctivitis and asthma caused by vine pollen: A case report

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Background: The vine (*Vitis vinifera*) is a cultivated plant that is found in some European and American countries. Its pollen gathers in small quantities during a short pollination period in the months of May and June. Allergy to vine pollen has not been previously documented.

Objective: We sought to describe a case report of allergy to vine pollen documented on the basis of anamnesis, cutaneous, provocation, and specific IgE determination tests.

Methods: An allergenic extract was obtained from collected *V. vinifera* pollens by aqueous standard procedures. Pollen counts and pollination periods of this and other common pollens in the area where the patient became symptomatic were studied. Cutaneous tests and the presence of specific IgE to the pollen extracts were performed by prick, CAP, and RAST techniques. Bronchial and conjunctival tests with the involved pollen extracts were also carried out to identify the sensitizing allergens. Five healthy subjects and 5 pollinic patients were used as control subjects and underwent the same tests.

Results: Skin prick test responses with vine pollen at different concentrations were positive for the studied patient and negative for the control subjects. Patient serum revealed a total IgE titer of 334 IU/mL and a specific IgE value of 1.3 PRU/mL (RAST class 2) to vine pollen. Bronchial and conjunctival provocation test responses were also positive when the patient was challenged with *V. vinifera* extract.

Conclusion: Exposure to the pollen of the vineyard plants (*V. vinifera*) can induce immunologic sensitization and rhinoconjunctivitis/asthma. (J Allergy Clin Immunol 1999;103:262-6.)

Key words: *Vitis vinifera*, vine pollen allergy, pollen counts, pollen extracts, provocation tests

The vine (*Vitis vinifera*) is a cultivated plant, which is found especially in the Mediterranean countries, the US, and Argentina. The climatic characteristics of Ciudad Real (province of Spain), with low rainfall (300 to 400 mm³ per year) and high temperatures, allow for the cultivation of vines that adapt well to the shallow, dry, limy soils in which the green foliage of the vine flourishes all through the summer. It is difficult for other plants to prosper in these conditions. The vineyards of Castilla-La Mancha form the area of highest concentration of this

Abbreviations used

BPT: Bronchial provocation test
PEFR: Peak expiratory flow rate
SPT: Skin prick test

plant existing in the world, representing 8% of the world area dedicated to the cultivation of vines.¹

The airborne nature of vine pollen has already been described by Jäger and Horak² in their description of pollens and hay fever in Austria in which they mention the small quantities of *V. vinifera* harvested in the Austrian lowlands. In 1977, a patient with rhinitis and asthma symptoms was described who had positive skin test responses to grass and vine pollens and intradermal reactions that were not confirmed through IgE and specific bronchial provocation with the allergens involved.³

The aim of this study was to describe a case report of allergy to vine pollen documented on the basis of anamnesis, cutaneous provocation, and specific IgE determination tests.

CASE REPORT

A 16-year-old girl had been treated in our clinic since 1994, after 3 years of nasal and ocular pruritus, conjunctival hyperemia, sneezing, nasal obstruction, coughing, tightness of the chest, and wheezing episodes. She had crises from April to July, and her symptoms worsened after spending time outside on dry and windy days. She received a diagnosis of seasonal rhinoconjunctivitis and bronchial asthma caused by sensitivity to grass, olive, and English plantain pollens and has been treated with preseasonal pollen immunotherapy for 3 years and, during symptomatic episodes, with nasal and bronchial budesonide, salmeterol, and salbutamol inhaled on demand.

During the last year and in May and June, her symptoms worsened after spending time in areas near vineyards. After the discrete response to previous treatment and because of the abundance of these crops in our province, we are considering the possible implications of vine pollen in the patient's clinical development.

The results of physical examination were normal at the time of the examination (November). A hemogram was performed, which showed 400 eosinophils/mm³. At the same time, a biochemical blood test and chest and paranasal radiographic tests were performed, with normal results.

Total IgE, calculated by the CAP method (Pharmacia Upjohn), was 786 kUA/L. The results of basal spirometry were normal: forced vital capacity of 334 (84% of the theoretical value) and FEV₁ of 3280 (97% of the theoretical value).

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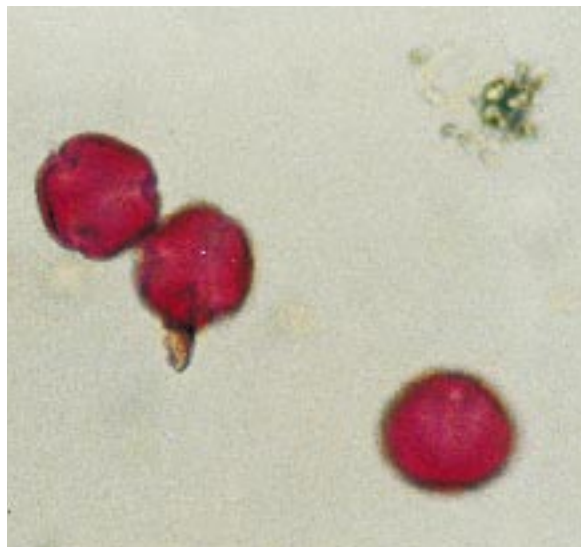


FIG 1. *Vitis vinifera* pollen viewed by optical microscopy.

METHODS

Preparation of *V vinifera* pollen extract

V vinifera pollen was collected in Ciudad Real province in the spring of 1997. The purity of the collected pollen was studied by microscopic particle count in 95% ethanol; 20 fields were examined at 400 $\times$ magnification, with a total of 740 counted pollen grains. Pollen identification was ascertained by electronic microscopy. Deffated pollen from the above-mentioned 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 5600g for 30 minutes, filtered through a 0.45- μ m pore diameter membrane, and dialyzed by ultracentrifugation in a Pelli-con System (Millipore Corp, Milford, Mass) with a 5000 d cut-off 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% of contaminant-mixed pollens and 9% of plant particles other than pollen. Pollen extract yielded 4.0 g of allergenic material per 100 g of pollen. Protein content was 10%, according to the Bradford method.⁴

Pollen count

Since 1987, continuous atmospheric pollen sampling has been conducted in Ciudad Real. The sampler was a Burckard 7-day volumetric spore trap (Hirst-type). The sampling airflow rate was 10 L/min, with the size of the orifice of a Hirst-type sampler being 14 $\times$ 2 mm. Pollens were caught on 24-mm wide transparent tape by a thin film of petroleum jelly. This tape was mounted on a cylinder rotating at a speed of 2 mm/hour. To study the pollens falling in a 24-hour period, a 48-mm sweep (2 mm $\times$ 24 hours = 48 mm) with the immersion lens was performed ($\times$ 10 ocular, $\times$ 100 objective, and microscope field diameter of 0.18 mm). The counts made in this 48-mm sweep, once multiplied by a conversion factor of 5.4, give us the average number of grains of pollen per cubic meter of air in 24 hours.

Skin prick test

The skin prick test (SPT) was performed according to the method of the Subcommittee on Skin Tests of the European Academy of Allergology and Clinical Immunology.⁵ Standardized lancets (Dome Hollister, Leverkusen, Germany) were used for SPTs.

The initial SPT was a standard aeroallergen battery, which

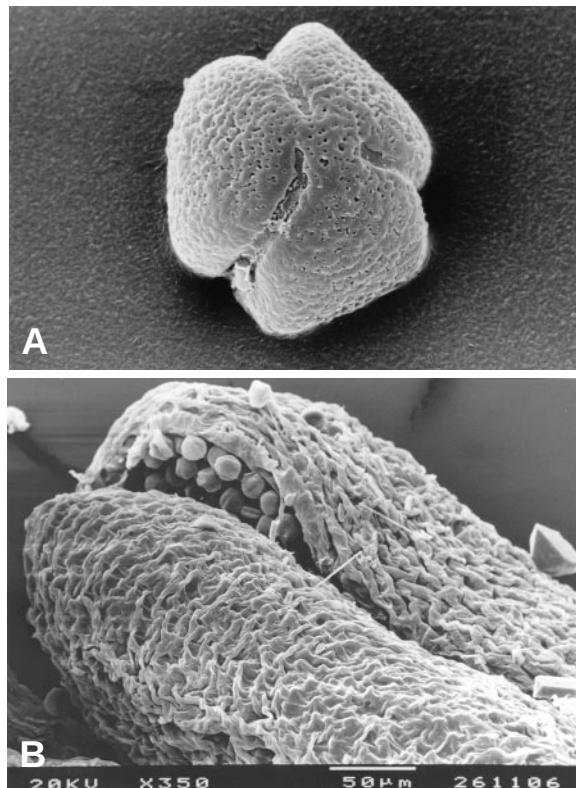


FIG 2. Scanning microscopy image of *Vitis vinifera* pollen (A) and pollen sac (B). Grains are tricolporate, isopolar, suboblate, or oblate and small (18 to 25 μ m). Exine are finely reticulated (perforate-microechinate). Microphotography kindly donated by M. Jerez and Dr J. Subiza.

included mites, Gramineae (*Lolium perenne*, *Dactylis glomerata*, and *Cynodon dactylon*), Oleaceae (*Olea europaea*), Chenopodiaceae (*Chenopodium album* and *Salsola kali*), Plantaginaceae (*Plantago lanceolata*), Compositae (*Artemisia vulgaris*), Fungi (*Alternaria alternata*, *Cladosporium herbarum*, and *Aspergillus fumigatus*), and cat and dog dander. This was followed by an SPT with vine pollen (*V vinifera*) at a concentration of 1, 2, and 5 mg/mL (wt/vol) supplied by BIAL-ARISTEGUI Laboratories (Bilbao, Spain). Histamine chlorhydrate at 10 mg/mL and phenolated glycerol saline solution were used as positive and negative controls, respectively.

To analyze possible irritative properties of the *V vinifera* extract, 10 control subjects (5 nonatopic subjects and 5 patients allergic to pollen) were also tested with this extract at a concentration of 5 mg/mL.

Specific IgE determination

Specific IgE to vine pollen was determined by the RAST technique. Solid-phase antigen was obtained by coupling the extract solution (10 mg/mL) to the 6-mm diameter cyanogen bromide-activated paper disks according to the method of Ceska and Lundqvist.⁶ RAST was performed according to the method of Wide et al,⁷ with Phadezym RAST reagents (Pharmacia Upjohn) according to the manufacturer's instructions. Specific IgE testing to other common local aeroallergens (*Lolium*, *Olea*, *Salsola*, *Plantago*, and *Alternaria* spp) was carried out by the CAP System (UniCAP, Pharmacia Upjohn).

Conjunctival provocation test

This test was performed according to the method of Jiménez et al.⁸ Briefly, the concentrated extract for the conjunctival provoca-

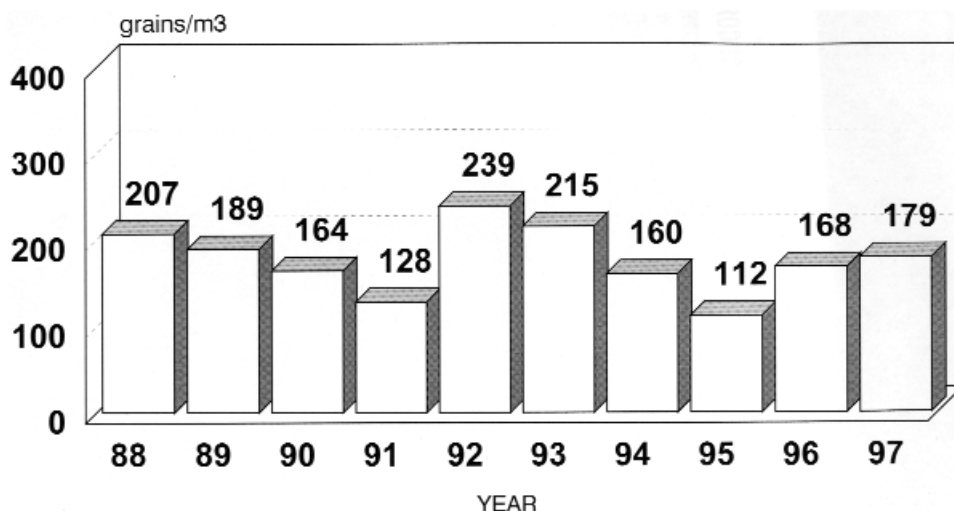


FIG 3. Airborne vine pollen in Ciudad Real from 1987 to 1997. Annual sums expressed as grains of pollen per cubic meter of air.

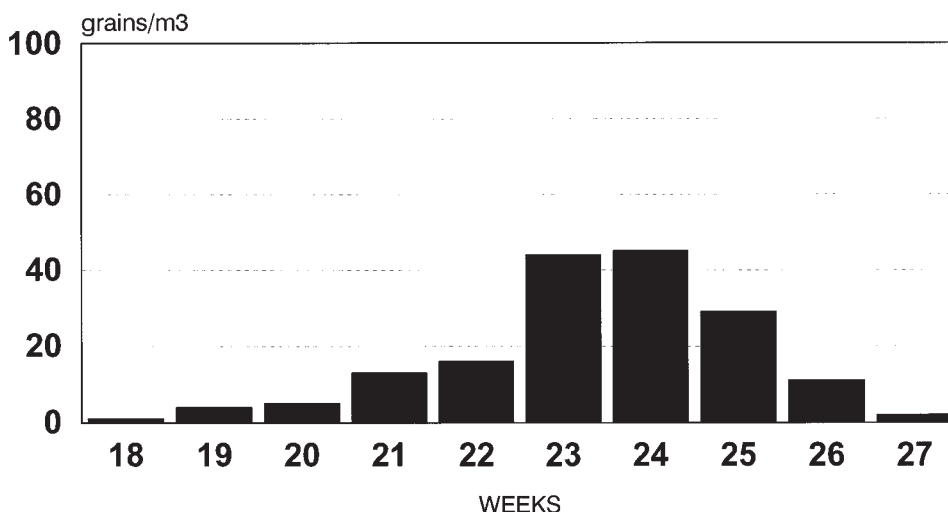


FIG 4. Vine pollen calendar in Ciudad Real, expressed as weekly averages, during 1987 to 1997.

tion test was prepared at 5 mg/mL. Serial dilutions were prepared at 0.1, 1, 2, and 5 mg/mL of *V. vinifera* pollen extract.

The test was started by administering 1 drop of diluent into the conjunctival sac. Ocular symptoms were monitored after 20 minutes. If no reaction was observed within 20 minutes, the next concentration (0.1 mg/mL) was applied to the other eye. The operation was repeated up to a concentration of 5 mg/mL unless a positive response was detected. The conjunctival provocation test response was considered positive under one of the following conditions: chemosis, erythema involving greater than 50% of the bulbar conjunctival area, or erythema involving less than 50% of the bulbar conjunctival area plus itching or epifora.⁹ Specificity controls were performed in 5 healthy subjects and 5 patients allergic to pollen by the same schedule. The latter showed the following symptomatology and specific sensitivities: patient 1, seasonal rhinoconjunctivitis and asthma caused by *Salsola*, *Plantago*, *Olea*, and grass pollens; patient 2, seasonal rhinoconjunctivitis and asthma caused by *Salsola*, *Olea*, and grass pollens; patient 3, seasonal rhinoconjunc-

tivitis caused by *Salsola*, *Olea*, and grass pollens; patient 4, seasonal rhinoconjunctivitis caused by *Salsola*, *Artemisia*, *Olea*, and grass pollens; and patient 5, seasonal rhinoconjunctivitis caused by *Plantago*, *Olea*, and grass pollens.

Bronchial provocation test

A nonspecific bronchial reactivity test with methacholine was done by using the procedure and concentrations proposed by Chatham et al,¹⁰ recording vital capacity, FEV₁, and peak expiratory flow rate (PEFR) values in an ST-250 spirometer (Fukuda Sangyo Co). Dilutions were administered with a Hudson 1720 nebulizer, with input and output flow rates of 7 L/min and 0.28 to 0.30 mL/min, respectively.

A specific bronchial provocation test (BPT) was performed with vine pollen extract, basically according to the method of Chai et al¹¹ but with some modifications. The patient was tested when asymptomatic and with an FEV₁ of greater than 80% of predicted normal value. He had not received any medication over the previous several

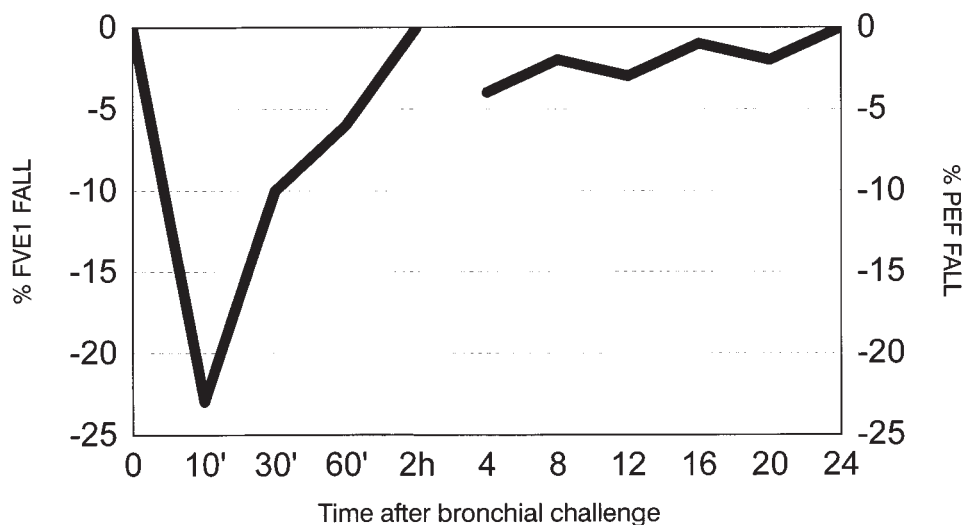


FIG 5. FEV₁ and PEF recordings after inhalation of 5 mg/mL (wt/vol) vine pollen extract.

days. A control challenge with PBS was carried out before antigen challenge. The patient was skin tested at concentrations of 1, 2, and 5 mg/mL vine pollen extract to determine a safe starting dose for the provocation test, and this concentration was 0.1 mg/mL extract (10-fold lower than the one that produced a 3 × 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. FEV₁ and PEF were measured after each inhalation and also 5, 10, 15, 30, and 60 minutes later. To assess the bronchial late response, the patient himself monitored PEF values in parallel to spirometry recordings by means of a portable Mini-Wright type peak flowmeter for up to 24 hours. A positive test response was defined as a fall in FEV₁ of 20% or in PEF of 25% from the postdiluent value during early or late determinations. Two atopic patients (numbers 1 and 2 described above) 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.

RESULTS

The vine pollen, which is oblate and tricolporate (Figs 1 and 2), gathers in small quantities (representing 0.8% of the pollens of Ciudad Real between the years 1987 and 1997) during a short pollination period (4 to 6 weeks) in the months of May and June (Figs 3 and 4).

Our patient showed the following SPT results when tested with the different concentrations of vine pollen extract: 1 mg/mL, 3 × 3 mm (longest and orthogonal wheal diameters); 2 mg/mL, 4 × 5 mm; and 5 mg/mL, 10 × 6 mm. SPT responses to a battery of commercially available common inhalants were positive to *Lolium* spp (10 × 10 mm), *Olea* spp (9 × 8 mm), *Plantago* spp (8 × 8 mm), and *Alternaria* spp (4 × 3 mm), with a histamine control response of 5 × 4 mm and no response to the saline control. SPT responses to vine pollen extract were negative in all control subjects, either nonatopic subjects or patients allergic to pollen who, on the other hand, had positive SPT responses to their respective sensitizing pollen extracts.

Specific IgE to vine pollen extract was positive (1.3 PRU/mL, RAST class 2). Specific IgE to common aeroallergens was positive to *Lolium* spp (>100 kUA/L, class 6), *Olea* spp (28.7 kUA/L, class 4), *Plantago* spp (23.5 kUA/L, class 4), and *Salsola* spp (12.4 kUA/L, class 3) but negative (class 0) to *Alternaria* spp.

The nonspecific BPT response to methacholine was positive (PD₂₀ = 50 AU). A BPT response to an antigen concentration of 5 mg/mL (wt/vol) was also positive, with recordings decreasing to 23% FEV₁ and 34% PEF, respectively (Fig 5). No delayed reaction was detected by peak flowmeter during the 24 hours after BPT.

Conjunctival provocation test responses were positive to an antigen concentration of 5 mg/mL. None of the control subjects had a positive response in conjunctival tests and BPTs, even with the maximum assayed dose (5 mg/mL).

DISCUSSION

In this article we describe the case of a patient who showed the symptoms for hay fever in the months of May and June, which worsened when in the open air of an area with a high density of vineyards. SPTs, specific IgE, and BPTs with vine pollen confirm the implication of this allergen in the patient's hay fever.

The climatic characteristics of Castilla-La Mancha, with low rainfall and extreme continental conditions, do not favor the cultivation of most crops. These poor, dry, semiarid lands extending over a great plain that hardly varies in altitude (eg, the provinces of Ciudad Real, Toledo, Albacete, and Cuenca) can only be made productive through the cultivation of vines. The wine-producing sector represents 20% of the agricultural activity (even more than cereal) and as a result plays a major role in the social and economic activity of the region, directly involving

70,000 families in the cultivation of vines and indirectly involving many more.

In spite of the extensive cultivation of vines in Mediterranean Europe, as well as Argentina and the US, it has not yet been described as a cause of hay fever. The vine pollen (medium-sized [20 μ m], oval, and tricolpated) is in the atmosphere of Ciudad Real for 4 to 6 weeks during the months of May and June. The aerobiologic study of the last 10 years in Ciudad Real (1987 to 1997) shows the scarce relevance of the participation of vine pollen, representing only 0.8% of the total pollen content, with peaks of a maximum of around 30 grains per cubic meter of air. These low levels of concentration coinciding with the period of pollination of grasses and olives have minimized the importance of the vine pollen, as Jäger and Horak² commented in their description of hay fever in Austria.

Hay fever is the most frequent pathology in Ciudad Real, as it is all over continental Spain, representing 90% of sensitization to airborne allergens.^{12,13} As we have previously pointed out, grass and olive pollens are the most important in terms of allergy, affecting 80% and 65%, respectively, of those people allergic to pollens. In third place we find Quenopodiaceae, with an incidence of 30%, related to the land characteristics and high summer temperatures, as found in the neighboring provinces of Toledo,¹⁴ Córdoba,¹⁵ and Sevilla.¹⁶ Other pollens play a more secondary role, as in the case of *Artemisia* or *Plantago* pollens. The last group consists of the pollens of *Pinus*, *Alnus*, or *Ulmus*, which, as a result of their low concentration in the atmosphere or their limited allergy-causing power, affect a minority of patients (range, 2% to 5%) but must nevertheless be taken into account when the symptoms of the patients correlate with the presence of these pollen groups in the atmosphere.¹⁷

The extensive cultivation of vines in our region can be expressed through the levels of pollen collected. The low quantities indicate that it is a pollen that is not easily airborne, but its capacity to produce an allergic reaction is demonstrated by the case we have described.

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