

Pollinosis related to *Zygophyllum fabago* in a Mediterranean area

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Received 19 January 2001; accepted in final form 23 April 2001

Key words: aerobiology, allergenicity, pollen calendar, pollinosis, *Zygophyllum fabago*

Abstract

Zygophyllum fabago is a herbaceous plant with airborne pollen found widely in the Murcia Region, in the Southeast of Spain. Although its allergenicity has been recently reported, little is known of its involvement in pollinosis. Aerobiological study and sensitization in pollinotics were measured using a Hirst volumetric trap. We have measured the atmospheric concentrations of this pollen and other allergenic pollen types in our region, between March 1993 and March 1997. *Z. fabago* pollen was collected for a morphometric study of the pollen grain, and a lyophilized extract was prepared for skin prick tests. We have conducted skin tests with different pollen types from our region and with *Z. fabago* in 1736 patients with symptoms suggesting pollinosis. The size of the pollen grain averages $15.17 \times 17.35 \mu\text{m}$. The pollination period extends from May to August, with a mean accumulated concentration of 448 grains/m³. Out of 1736 pollinotics, 263 (15.15%) showed a positive skin test for *Z. fabago*, 6 were monosensitized and 257 were sensitized to other common pollen types from our region. Specific IgE to *Z. fabago* was equal or higher than 0.35 ku/l in 86.56% of sensitized patients. Chenopodiaceae pollinate during spring and autumn and sensitize a larger number of patients; Urticaceae reach the highest pollen concentrations and for a longer period but are not the primary cause of pollinosis. This study shows that *Z. fabago* pollen becomes airborne, elicits an IgE response and, like other pollens, contributes towards triggering allergic symptoms. It should therefore be considered a relevant allergen and accordingly be included in skin test procedures.

1. Introduction

Aerobiological studies give us qualitative and quantitative information about the common pollen types in the atmosphere of a certain geographical area, indicating the presence of particles potentially responsible for allergic manifestations (Frankland, 1991). However, they cannot replace the experimental confirmation of the human response. Therefore, as more is learnt about the palinological composition of the local atmosphere, new agents are reported as the cause of allergy symptoms (Keynan et al., 1997). Identification of the allergens responsible for allergic

reactions will complement the existing studies that can provide us with a deeper and more accurate knowledge of the causes of pollinosis.

The incidence of allergies due to airborne biological material has increased in recent years without a clear reason being known (Sibbald and Rink, 1991). Clinically, they manifest by means of rhinoconjunctivitis and asthma, and usually in spring although it is not uncommon for patients to have symptoms in summer or early autumn. It occurs in patients sensitive to one or more families of pollens during the blossoming period.

Significant amounts of *Z. fabago* pollen grains have been found in the atmosphere of our region, which may be involved in the development of allergy symptoms (Belchí et al., 1997).

Z. fabago belongs to the Zygophyllaceae family, Order Sapindaceae (Cronquist, 1981). It is a ramose, perennial plant with 50–80 cm high stalks and oval-shaped, slightly fleshy leaves, which are light green in colour and hairless. The flowers are arranged on the axillae of the leaves and each consists of five petals: cream-coloured on the top and orange at the bottom. The plant has ten protruding stamens which facilitate dispersion of the pollen grains by the wind. It blossoms in spring and summer, from May onwards, and grows on building lots, among rubble, in stone quarries, on steep embankments and on low-lying waste land, which is why it is commonly found in rural areas and close to urban areas. It is abundant in coastal zones and uncommon in areas away from the coast, being typical of the Middle East, North Africa and the more arid parts of the Mediterranean (Font, 1993).

In this article we study *Z. fabago* pollen and its aerobiology. We also study the sensitization frequency of this and other allergenic pollens in our geographical area, together with the rate of sensitization caused in our area of study.

2. Materials and methods

2.1 *Z. fabago* pollen aerobiology

The aerobiological data between March 1993 and March 1997 were obtained using a pollen trap (Lanzoni VPPS 2000, Italy) placed on the roof of Cartagena railway station, located at 0°59' W longitude, 37°36' N latitude and at a height of 10 m, without gardens or parks nearby. The area around the pollen trap is urbanized, located between the Mediterranean sea and low hills, has a phytoclimatic position with typical Mediterranean vegetation.

The air was sucked in at a flow rate of 10 l/min onto a Melinex tape coated with a thin film of silicone diluted in carbon tetrachloride and acetone. After sampling, the tape was cut into 48 mm segments, each corresponding to 24 hours, which were placed on a 76×26 mm microscope slide and mounted in glycerine jelly containing fuchsin as a staining agent. Four rows per slide were counted using an Olympus BH-2 microscope with D-Plan optics and 500x magnification (10x ocular lens and 50x immersion objective

lens) and the number of pollen grains per cubic meter of air was calculated according to Moreno-Grau et al. (1995).

2.2 Patients, symptoms and skin prick tests

In the period between July 1994 and September 1996, patients in our department with symptoms suggesting rhinoconjunctivitis or pollen asthma were selected, both living in a rural or urban area. According to the guidelines of the subcommittee on skin tests of the European Academy of Allergy and Clinical Immunology (EAACI skin test subcommittee, 1993), we performed skin prick tests with a series of common pollens from this region (Ifidesa Aristegui, Spain), which included: *Olea*, Poaceae, *Plantago*, *Artemisia*, *Parietaria*, *Chenopodium*, *Salsola*, *Mercurialis annua*, *Pinus*, *Cupressus*, *Platanus*, *Helianthus*, *Taraxacum*, *Rumex*, *Chrysanthemum* and a *Zygophyllum* extract prepared in our laboratory. A group of 20 non-atopic patients was tested with *Zygophyllum* as a control to ensure that the extract for the skin test was not irritating.

2.3 Pollen extract

The purity of the collected pollen was determined using optic microscopy. Ether ethylic defatted pollen was extracted twice with magnetic stirring (2 hours at 4 °C) in 0.05 M phosphate buffer, pH 8.0, at 6.6% (w/v). The extract was clarified by centrifugation at 5,600 g for 30 min, filtered through a 0.45 µm pore diameter membrane and dialyzed by ultracentrifugation in a Pellicon system (Millipore corp., Milford, Mass) with a 5,000 dalton cut-off. The dialyzed extract was sterilized by filtration through a 0.22 µm pore diameter membrane, lyophilized for storage at 4 °C and later recovery to perform skin prick tests at a concentration of 2.3 mg/ml.

2.4 Test for pollen-reactive IgE antibodies

Solid-phase antigen was obtained by coupling the extract solution (10 mg/ml) to 6 mm diameter CNBr-activated paper discs according to Ceska et al. (1972). Enzyme Immuno Assay (EIA) was performed with phadezym reagents (Kabi Pharmacia Diagnostics AB) according to the manufacturer's instructions (Wide et al., 1967). A group of 10 atopic patients not sensitized to *Z. fabago* was used as a control.

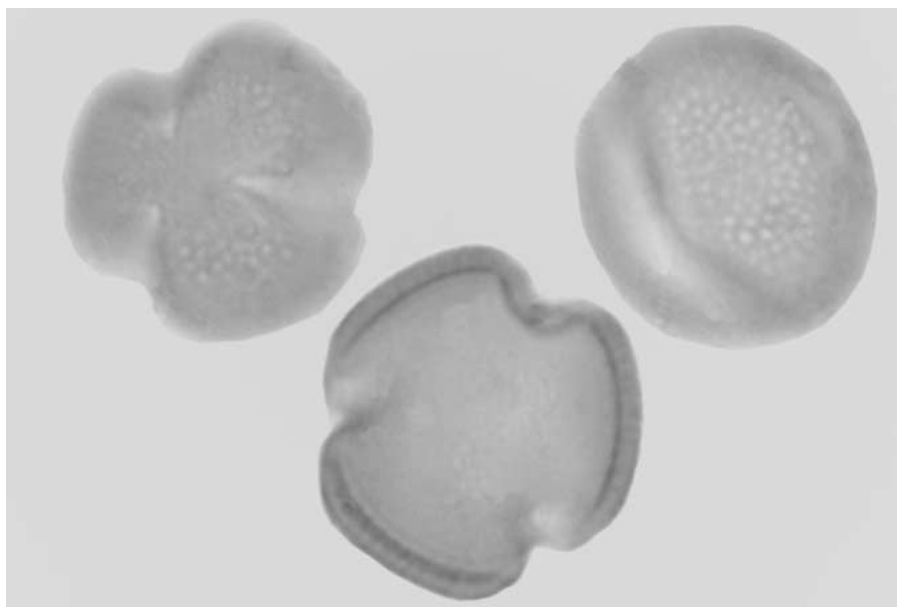


Figure 1. Microscopic image of *Zygophyllum fabago* pollen grains.

3. Results

The pollen grains are small in size (Polar axis: $15.17 \pm 0.19 \mu\text{m}$, Equatorial axis: $17.35 \pm 0.23 \mu\text{m}$), isopolar, suboblated, tricolporated and reticulated. The octoaperture is large, the apocolpium is small and the endoaperture is longitudinally elongated (Figure 1).

During the period between March 1993 and March 1997, a total yearly average of 18119 pollen grains/ m^3 per year was reached, of which 15438 corresponded to taxa with a proven allergenicity, accounting for 85% of the yearly count of airborne pollen. The *Zygophyllum* pollen reached 448 grains/ m^3 /year (Figure 2). Table 1 shows the number of grains per year for taxa of allergological interest.

In the period between July 1994 and September 1996 we diagnosed 1736 pollinic patients, of whom 1573 (90.62%) were polysensitized and only 163 (9.38%) monosensitized (Table 2). No patient was monosensitized to *Pinus*.

Out of the 1736 patients, 1076 (61.98%) lived in urban areas and 660 (38.02%) in rural areas, 832 males and 904 females with a mean age of 31.23 ± 11.48 years (range: 5–62 years). Rhinoconjunctivitis was diagnosed in 1284 (73.96%) and rhinoconjunctivitis plus asthma in 452 (26.04%).

Two hundred and sixty-three patients were sensitized to *Z. fabago* pollen, their characteristics being

Table 1. Pollen concentration (grains/ m^3) of the allergenic taxa registered in Cartagena from March 1993 to March 1997

Taxa	Period			
	1993–94	1994–95	1995–96	1996–97
<i>Artemisia</i>	516	160	135	345
Cupressaceae	2873	2218	1361	2959
Cheno.-Amar.	4166	2308	2851	2027
Liguliflorae	23	29	16	22
(Asteraceae)				
Poaceae	1370	632	670	685
<i>Mercurialis</i>	23	10	1	49
<i>Olea</i>	2058	1968	1119	795
<i>Pinus</i>	1685	392	564	433
Plantaginaceae	772	363	424	150
<i>Platanus</i>	660	418	318	126
<i>Rumex</i>	123	78	62	63
Tubiflorae	89	31	13	11
(Asteraceae)				
Urticaceae	9511	4746	4222	3540
<i>Zygophyllum</i>	397	403	455	538

shown in Table 3. Only 6 patients (2.28%) proved to be monosensitized to this pollen type, and all of them with rhinoconjunctivitis symptoms. Two hundred and fifty seven patients (97.72%) were polysensitized to other pollens types: 185 to *Olea*, 149 to Poaceae, 48 to *Plantago*, 98 to *Parietaria*, 73 to *Artemisia*, 168

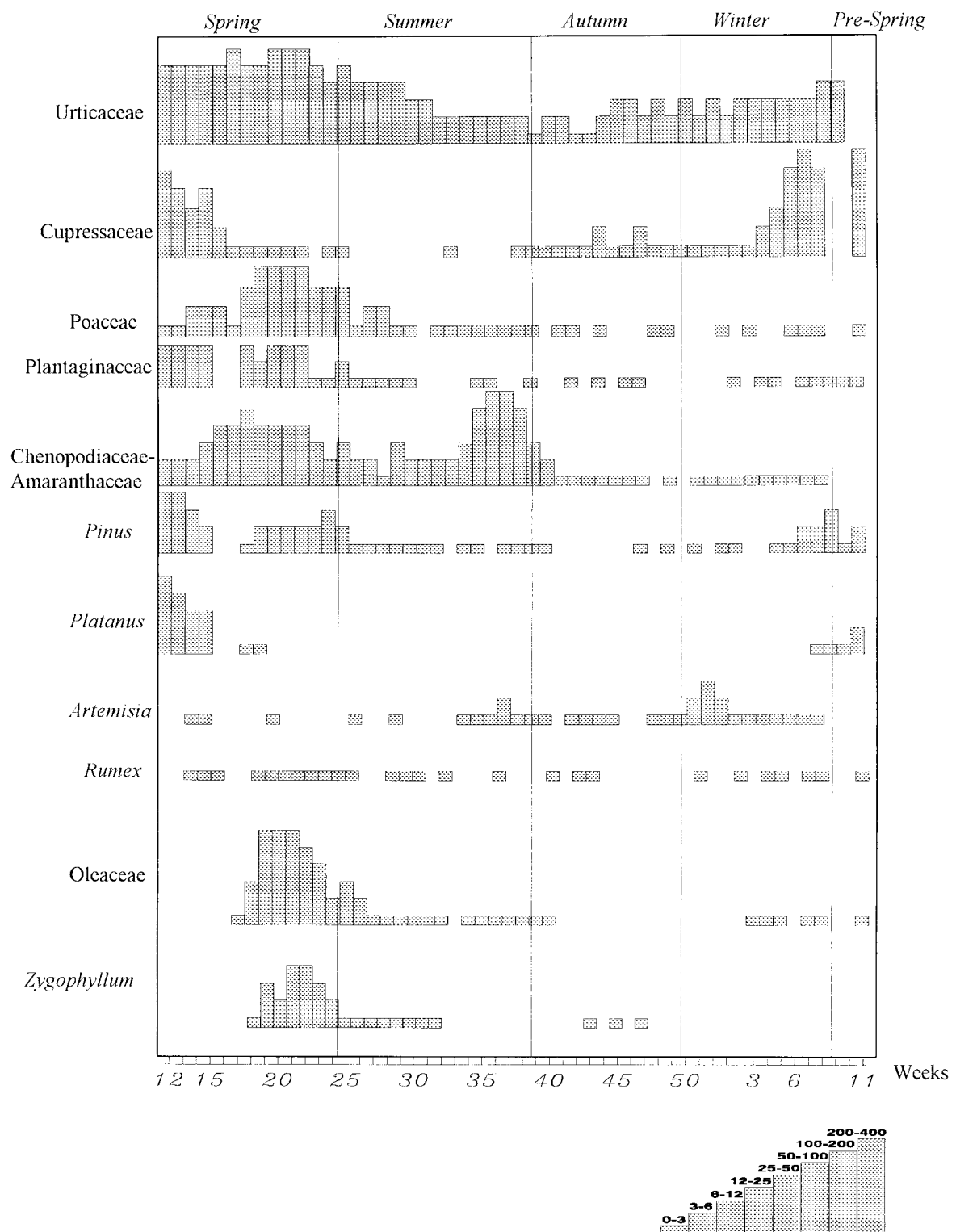


Figure 2. Pollen calendar of the allergenic taxa registered in Cartagena (log scale) from March 1993 to March 1997.

Table 2. Pollen concentration of the common taxa and frequency of sensitization in 1736 pollinoyivd

Taxa	Average of 1993–97 (grains/m ³)	Skin prick test positivity (n = 1736)			
		Polysensitized patients (n = 1573)		Monosensitized patients (n = 163)	
<i>Artemisia</i>	289	443	28.09%	15	9.20%
Cupressaceae	235	49	3.11%	1	0.61%
Cheno.-Amar.	2838	1029	65.41%	29	17.79%
Poaceae	839	808	51.36%	18	11.04%
<i>Mercurialis</i>	21	189	12.01%	3	1.84%
Oleaceae	1485	1061	67.45%	39	23.93%
<i>Pinus</i>	768	7	0.44%	0	0.00%
<i>Plantago</i>	427	252	16.02%	2	1.23%
<i>Platanus</i>	380	30	1.90%	2	1.23%
<i>Rumex</i>	87	37	2.35%	0	0.00%
Urticaceae	5504	586	37.25%	48	29.45%
<i>Zygophyllum</i>	448	257	15.15%	6	3.68%

Table 3. Features of patients skin positivity to *Zygophyllum fabago* pollen extract (n = 263)

Monosensitized		6 (2.28%)
Age		25.33±7.24 years
Gender		4M/2F
Habitat	Rural	6 (100%)
	Urban	0 (0%)
Symptoms	Rhinoconjunctivitis	6 (100%)
	Rhinoconjunctivitis + asthma	0 (0%)
Polysensitized		257 (97.72%)
Age		31.02±9.76 years
Gender		138M/119F
Habitat	Rural	98 (38.14%)
	Urban	159 (61.86%)
Symptoms	Rhinoconjunctivitis	184 (71.59%)
	Rhinoconjunctivitis + asthma	73 (28.41%)

to *Chenopodium*, 40 to *Mercurialis*, 4 to *Platanus*, 82 were polysensitized to other pollen types. None of these patients was sensitized to *Cupressus* or *Pinus*.

All the subjects from the control group had a negative skin prick test with *Zygophyllum* extract.

Specific IgE to *Zygophyllum* pollen was found in only 67 patients (levels ranging from 0.35 to more than 17.5 ku/l). EIA class 4 was found in 6 sera (8.95%), two of them were from monosensitized patients, class 3 in 17 (25.38%), class 2 in 25 (37.32%), and class 1 in 10 (14.92%). Specific IgE was negative in only 9 sera (13.43%). The highest EIA values generally corresponded to patients who had larger skin tests. The control group had values of <0.35 ku/l.

4. Discussion

Z. fabago has not yet been considered as an aero-allergen despite its wide distribution and concentrations in the atmosphere. We have not found any other study in which this pollen is considered allergenic. In a previous study, our group showed that this pollen sensitizes the exposed population and, by specific provocation, is responsible for causing allergic symptoms (Belchí et al., 1998).

Pollen counts carried out during this study showed *Zygophyllum* pollen reaches relatively high concentration levels in the atmosphere, which accounts for its potential allergenicity together with its morphological characteristics.

The mean concentration of this pollen was 448 grains/m³, a similar value to those reported for other allergenic pollens (Ariano et al., 1993). This value, compared to the total pollen count, represents a mean of 2.9%, and 6.1% of the total pollens in their pollination period.

The present study was designed as a prospective trial to detect sensitization to *Z. fabago*. Sensitization to this pollen was detected in 263 (15.15%) of the 1736 considered pollinotics, a comparable percentage to other allergenic pollen values reported (Fernandes and Menezes, 1995). 61.86% of sensitized patients came from the urban area, meanwhile only 38.14% came from a rural zone, although this plant has proven to be widely distributed in our region. However, the 6 monosensitized patients (2.28%) came from rural areas, perhaps due to a greater exposure to *Z. fabago* pollen.

One of the most outstanding features of the aerobiology in our area is the year-round presence of Chenopodiaceae-Amaranthaceae and Urticaceae pollen types. Two airborne pollen seasons were clearly identified for Chenopodiaceae-Amaranthaceae, being the most representative taxon of late summer and early autumn. Another blossoming peak occurs during the spring time, although the number of species is much lower. This is probably the cause of 60.94% of our patients being sensitized to this pollen type.

Although Urticaceae are present the whole year, their maximum blossoming peak occurs during spring, when they represent 30% of the total pollen count. The number of pollen grains per year reaches twice the value obtained by Chenopodiaceae-Amaranthaceae. However, patients are less sensitized to Urticaceae than to Chenopodiaceae, although it is the main taxon causing monosensitization.

Oleaceae have a well-defined spring pollination period, accounting only for 10% of the total allergenic pollen. However, allergenicity of grass pollen is well known, being the most important cause of pollinosis in early summer all over Europe (Spieksma, 1994). In our research, they sensitized up to 51% of our patients.

Cupressaceae represents the most abundant taxon in the pre-spring season and at the end of autumn, due to *Tetraclinis* and *Sabina* blossoming. They reach high concentrations in a short pollination period and sensitize only 3% of the pollinotic population. On the other hand, *Artemisia*, which blossoms during autumn and winter, depicts low pollen concentrations but a high sensitization rate (26.38%). Everyone sensitized to other Asteraceae are also sensitized to *Artemisia*, probably one of the most powerful aeroallergens. *Pinus* pollen also presents moderate concentrations and a very low degree of sensitization, which could be due to its morphology, aerobiology and pollen grain size (Solomon and Mathews, 1992).

Z. fabago, like other pollen types (*Mercurialis*, *Plantago*, *Rumex*, *Platanus*), does not attain very high concentrations. This could be the main reason why they are unlikely to cause pollinosis by themselves. Its pollination period coincides totally or partially with that of other taxa and considerably increase the total quantity of allergenic pollens.

Therefore, we consider that *Z. fabago* helps to increase the allergenic pressure to which polysensitized pollinotic are exposed, such that low levels of one taxon may not trigger allergy symptoms, but the combined action of low levels of various taxa will probably cause allergy symptoms in these patients (Salomon, 1984).

5. Conclusions

This study shows that *Zygophyllum fabago* pollen is an aeroallergen and triggers an allergic response. We therefore consider it of interest to study its atmospheric distribution and include it in the pollen battery for skin tests.

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