

Zygophyllum fabago L: A new source of allergenic pollen

Juan Belchí-Hernández, MD,^a Stella Moreno-Grau, PhD,^b Javier Bayo, BSc,^b
Caridad Rosique, BSc,^b Borja Bartolomé, PhD,^c and Jose M. Moreno, BSc^b
Murcia, Cartagena, and Bilbao, Spain

Zygophyllum fabago is a widespread herbaceous plant in the Mediterranean area that belongs to the Zygophyllaceae family (order of the Sapindaceae). The genus *Zygophyllum*, with about 90 species, grows mainly between northern Africa and central Asia.¹ The pollen is both insect and wind borne and the plant has a flowering period that lasts from May to August. The pollen grains are isopolar, suboblate, tricolporate, and reticulated. The octoaperture is large, the apocolpium is small, and the endoaperture is longitudinally elongated.²

An aerobiologic study in Cartagena, Murcia, Spain, found that *Zygophyllum* pollen grains accounted for 6.06% of the total pollen during the period of pollination. The presence of the pollen in the atmosphere may be related to the exertive disposition of the stamen and the abundant biomass of this plant.

The only previous mention of the Zygophyllaceae family as a possible inducer of allergic diseases was by Small and Smell in 1946.³ We therefore conducted a prospective study to determine the allergenicity and aerobiologic characteristics of this pollen.

METHODS

Pollen characteristics and pollen season

To determine the size of *Zygophyllum fabago* pollen grains, we collected dehiscent anthers in the Murcia region during the springs of 1994 and 1995. After acetylating and mounting the pollen grains in glycerine jelly, we measured 100 grains per slide with use of an image analysis apparatus equipped with a Micron Imaging Processing software package (MIP Microm, Spain).

Airborne pollen was collected by a Hirst 7-day recording volumetric trap (Lanzoni VPPA 2000, Italy) between January 1993 and December 1995. The trap was installed, exposed to all winds, on the roof of Cartagena railway station, located at 0 degrees, 59 minutes west longitude and 37 degrees, 36 minutes north latitude and at a height of 10 meters, with no nearby gardens or parks. The number of pollen grains per cubic meter of air was calculated according to the method of Moreno-Grau et al.⁴

Abbreviations used

CPT: Conjunctival provocation test
SPT unit: Skin prick test unit

Pollen extract

We estimated the purity of the collected pollen to be 99% by optic microscopy. Pollen extract was prepared with the standard weight/volume method. We determined the percent protein of the pollen extract by the Bradford method.

Patients and symptoms

Between July 1994 and September 1995 we selected 648 consecutive patients from rural and urban areas seen by our department with rhinoconjunctivitis or pollen asthma. The first 34 patients with a positive skin test response to *Zygophyllum fabago* were selected to undergo specific IgE determination and a conjunctival provocation test (CPT).

Skin tests

Skin prick testing was done according to the guidelines of the subcommittee on skin tests of the European Academy of Allergology and Clinical Immunology⁵ with pollen extract diluted in 0.9% NaCl, 0.5% phenol, and 50% glycerol solution at concentrations of 0.1, 1, 5, 10, and 50 mg/ml. The mean wheal area was plotted as a function of the allergen concentration in a log-log system, and linear regression was done. The allergen concentration that elicited a wheal equal to the wheal produced by histamine 10 mg/ml was defined as 1 skin prick test unit (SPT unit). One SPT unit was found to be equivalent to 2.3 mg/ml of allergen extract.⁶ This concentration was used to establish the maximum dose of allergen to be used in provocation tests.

Prick tests were done with a battery of mites, molds, dander, and pollens (Ifidesa Aristegui, Spain), which included *Olea*, Gramineae, *Plantago*, *Artemisia*, *Parietaria*, *Chenopodium*, *Sal-sola*, *Mercurialis*, *Robinia*, *Pinus*, *Cupressus*, *Platanus*, *Heli-anthus*, *Taraxacum*, *Rumex*, and *Chrysanthemum*.

Serum specific IgE assay

Solid-phase antigen was obtained by the method of Ceska and Lundqvist.⁷ RAST was done by the method of Wide et al.⁸ with Phadezym Trazer reagents (Kabi Pharmacia Diagnostics AB). As a control we tested 10 serum samples from patients allergic to *Olea*.

CPT

The CPT was done according to the method of Moller et al.⁹ The criteria for a positive test result were congestion of the conjunctival mucosa and itching and watering of the eye and nose. A negative control was done by administering one drop of the diluent into the right conjunctival sac. The range of extract

From ^athe Department of Allergy, University Hospital J. M. Morales Meseguer, Murcia; ^bDepartamento de Ingeniería Química, Escuela Politécnica Superior de Cartagena, Universidad de Murcia; and ^cI + D Department IFIDESA, Bilbao, Spain.

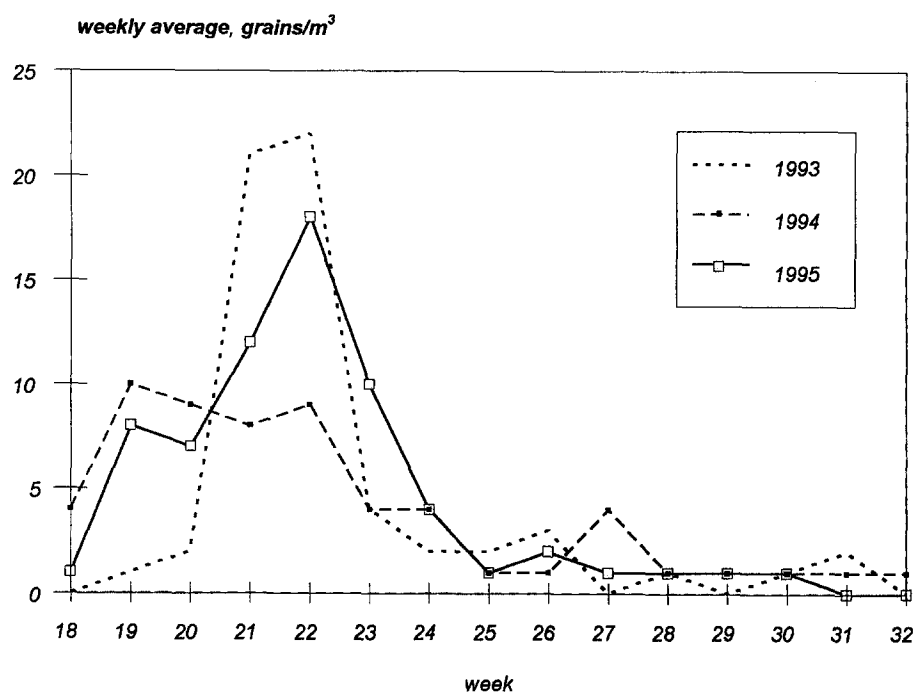
Received for publication Feb. 26, 1996; revised Oct. 28, 1996; accepted for publication Oct. 31, 1996.

Reprint requests: Juan Belchí-Hernández, MD, C/Los Torres No. 11, 1^a Esc. 7^o C, 30005 Murcia, Spain.

J Allergy Clin Immunol 1997;99:493-6.

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/1/79228



Zygophyllum POLLEN SEASON					POLLEN CONCENTRATION		
Year	1st day	last day	MAX	Duration (days)	MAX	ACUM*	%
1993	May, 25	Aug, 07	Jun, 02	75	58	399/8536	4.67
1994	May, 05	Aug, 09	Jun, 01	97	25	405/5831	6.95
1995	May, 04	Jul, 13	Jun, 03	71	43	440/6193	7.11
Mean				81	42	415/6853	6.06

*Z. *Fabago* pollen counts in the pollen season / total pollen counts in this period

FIG. 1. *Zygophyllum fabago* pollen season and concentrations. MAX, Maximum; ACUM, Accumulative.

TABLE I. Morphometric analysis of *Z. fabago* samples

Parameter	X (μm)	Standard deviation (μm)	Minimum (μm)	Maximum (μm)
Polar axis	15.17 ± 0.19	0.54	14.23	16.33
Equatorial diameter	17.35 ± 0.23	0.64	15.91	19.85

dilutions was 0.001, 0.01, 0.1, and 1 SPT unit. As a control we tested 10 patients seen with a respiratory allergy to *Olea* pollen.

RESULTS

Table I shows the morphometric analysis of the pollen and the statistical parameters. Fig. 1 depicts pollen concentrations during the 1993 to 1995 *Zygophyllum*

fabago seasons. Table II shows accumulated pollen per cubic meter for the flowering periods in 1993, 1994, and 1995 for both this and other potentially allergenic taxa.

Among the 648 patients tested with *Zygophyllum* extract, 114 patients (17.59%, 61 men, 53 women, age 22.7 ± 6.8 years) had development of a wheal greater than 5 mm. Of the 114 patients with positive skin test

TABLE II. Pollen concentrations of other allergenically important taxa in the *Z. fabago* pollination period

	1993		1994		1995	
	Grains/m ³	%	Grains/m ³	%	Grains/m ³	%
<i>Zygophyllum</i>	399	4.67	405	6.89	440	7.11
Chenopodiaceae	846	9.95	525	9.00	595	9.61
<i>Olea</i>	1150	13.47	1422	25.79	1117	18.04
Poaceae	807	9.45	471	8.08	452	7.30
Urticaceae	3098	36.29	1680	28.81	1900	30.68
<i>Quercus</i>	956	11.20	204	3.50	757	12.22
<i>Ligustrum</i>	255	2.99	185	3.17	36	0.58
Asteraceae	36	0.42	33	0.57	11	0.18
Partial sum	7547	88.41	4925	84.46	5308	85.71
Season total	8536		5831		6193	

results, 76 (62%) lived in rural areas and 48 (38%) in urban areas, and all had a seasonal pattern of allergic symptoms. We found only 2 patients with monosensitization, and both had symptoms of rhinoconjunctivitis (recorded on a diary card) only during the flowering period of *Zygophyllum fabago*. The remaining 112 had polysensitization to other pollens as follows: *Olea* 70.49%, Gramineae 57.12%, *Plantago* 19.59%, *Parietaria* 37.8%, *Artemisia* 32.78%, *Chenopodium* 63.93%, *Salsola* 70.49%, *Mercurialis* 16.39%, *Platanus* 1.6%, *Robinia* 3.26%, *Cupressus* 4.9%, *Pinus* 1.6%, *Helianthus* 14.75%, *Taraxacum* 3.26%, and *Chrysanthemum* 14.75%. Forty-two percent reported rhinoconjunctival symptoms plus asthma, and 58% reported only rhinoconjunctival symptoms.

The CPT was done and specific IgE to *Zygophyllum* pollen was determined in 34 patients. A RAST class 4 result was found in 3 sera, class 2 in 20, class 1 in 6, and class 0 in 5. The CPT result was negative in 4 patients and positive in 30 patients. Of the patients with negative CPT results, 3 had RAST class 0 results and 1 patient had class 1 results. Two of the patients with RAST class 0 results, 4 patients with class 1 results, and all patients with class 2 or higher results had positive CPT results. The control group had RAST class 0 results and a negative response to the CPT.

DISCUSSION

Pollen counts showed that *Zygophyllum* pollen reached relatively high concentration levels in the atmosphere, which with the morphologic characteristics of this pollen accounts for its potential allergenicity. Despite widespread distribution and atmospheric levels, *Zygophyllaceae* pollens have not been considered as aeroallergens. This may be because the plant is pollinated by insects and because of the lack of knowledge of how different airborne pollens affect persons who are likely to be sensitized. However, when certain pollens enter a warm, moist environment, such as human mucosa, they may increase protein synthesis in the intine and uncus, thus increasing antigenic power and load.¹⁰ We do not know of any study of the allergenicity of this pollen. The present study was designed, therefore, to

detect sensitization to *Zygophyllum* pollen. Sensitization was detected in 114 (17.59%) of the 648 patients with a positive skin test result, a percentage that is similar to that found for other allergenic pollens. To date we have only found 2 patients with monosensitization, which implies that *Zygophyllum* pollen is solely responsible for their symptoms. In the rest of the patients this sensitization would act rather as an additive to the response.

Specific IgE determinations and CPT results showed a good correlation with cutaneous sensitivity. These results are similar to those found in other studies on new allergenic pollens, and the results of CPT establish a reasonable relationship between exposure and symptoms.¹¹⁻¹³ This plant is not phylogenetically related to other allergenic weeds in our area, and cross-reactivity with other plants is unlikely.

Zygophyllum pollen could increase the allergenic load in Mediterranean areas and must be considered as a cause of respiratory allergic disease. Our further aims are to conduct an immunologic study to identify pollen antigens and determine whether cross-reactivity exists.

We thank Maria Teresa Zamora and Beneta Iniesta for assistance in the performance of this study.

REFERENCES

1. Cronquist A. An integrated system of classification of flowering plants. New York: Columbia University Press, 1981.
2. Suarez-Cervera M, Marquez J, Moreno-Grau S, Bayo J, Rosique C, Belchí J. Ultrastructure et immunocytochimie du pollen de *Zygophyllum fabago*: palynologie and ahaugements bloiaux. In: Abstracts of 14 Symposium Association de Palynologues du Langue Francaise 1995. Paris: Association de Palynologues du Langue Francaise. Vol des Resumes, 100.
3. Lewis WH, Winay P, Zenger VE. Airborne and allergenic pollens of North America. Baltimore: Johns Hopkins University Press, 1983: 556.
4. Moreno-Grau S, Bayo J, Rosique C, Moreno JM, Moreno J, Takahashi Y. Concentration of atmospheric pollen in Cartagena (Spain) from March 1993 to March 1994. Jpn J Palynol 1995;6:565.
5. EAACI Skin Test Subcommittee. Allergens standardization and skin tests. Allergy 1993;48(suppl):15-20.
6. Malling HJ. Quantitative skin prick testing. Allergy 1987;42:196-204.
7. Ceska M, Lundqvist U. A new and simple radioimmunoassay method for the determination of IgE. Immunochemistry 1972;9: 102-5.

8. Wide L, Bennich H, Johansson SGO. Diagnosis of allergy by an in vitro test for allergen antibodies. *Lancet* 1967;2:1105-7.
9. Moller C, Bjorksten B, Nilsson G, Dreborg S. The precision of the conjunctival provocation test. *Allergy* 1984;39:37-41.
10. Casas-Ferrer C. Caracterización inmunocitoquímica de los granos de polen de *Parietaria judaica* L. (Urticaceae) [Thesis]. Barcelona: Universidad de Barcelona, 1995:78.
11. Ariano R, Panzani RC, Chiapella M, Augeri G. Respiratory allergy to the pollen of *Mercurialis annua*. *Ann Allergy* 1993;70:249-54.
12. Fernandes L, Menezes Mesquita A. *Anacardium occidentale* (cashew) pollen allergy in patients with allergic bronchial asthma. *J Allergy Clin Immunol* 1995;95:501-4.
13. Garcia-Ortega P, Martinez J, Martinez A, Palacio R, Belmonte G. *Mercurialis annua* pollen: a new source of allergenic sensitization and respiratory disease. *J Allergy Clin Immunol* 1992;89:987-93.

Bound volumes available to subscribers

Bound volumes of The Journal of Allergy and Clinical Immunology are available to subscribers (only) for the 1997 issues from the Publisher, at a cost of \$88.50 for domestic, \$116.10 for Canadian, and \$108.50 for international subscribers for Vol. 99 (January-June) and Vol. 100 (July-December). Shipping charges are included. Each bound volume contains a subject and author index, and all advertising is removed. Copies are shipped within 30 days after publication of the last issue in the volume. The binding is durable buckram with the journal name, volume number, and year stamped in gold on the spine. *Payment must accompany all orders.* Contact Mosby-Year Book, Inc., Subscription Services, 11830 Westline Industrial Dr., St. Louis, MO 63146-3318; phone 1 (800) 453-4351 or (314) 453-4351.

Subscriptions must be in force to qualify. Bound volumes are not available in place of a regular journal subscription.

The use of IgE immunoblotting as a diagnostic tool in *Anisakis simplex* allergy

Milagros García, MD,^a Ignacio Moneo, MD, PhD,^b Maria Teresa Audicana, MD,^a María Dolores del Pozo, MD,^a Daniel Muñoz, MD,^a Eduardo Fernández, MD,^a Juan Díez, MD,^a Miguel A. Etxenagusia, MD,^a Ignacio J. Ansotegui, MD, PhD,^a and Luis Fernández de Corres, MD, PhD^a

Vitoria-Gasteiz (Basque Country) and Madrid, Spain

Background: The fish parasite *Anisakis simplex* is the etiologic agent of anisakiasis and induces IgE-mediated reactions. Skin prick tests (SPTs) and the measurement of specific IgE to *A. simplex* were, in our experience, not valid tools with which to discriminate between allergic and nonallergic patients because many control subjects also had positive results.

Objective: The study was carried out to assess the usefulness of IgE immunoblotting in the diagnosis of allergy to *A. simplex*.

Methods: We have studied 61 patients with acute symptoms of urticaria, angioedema, or anaphylaxis and positive specific IgE to *A. simplex*. According to the anamnesis, time interval between ingestion of fish and clinical onset of symptoms, and exclusion of other causes of allergy, three different groups of patients were established: group A (allergic), group NA (non-allergic), and group D (doubtful). Fifty-one healthy donors were included as control subjects (group C). IgE immunoblotting with *A. simplex* whole-body extract was performed in all patients and control subjects.

Results: Four patterns of immunoblotting were observed: type 1, with a group of several bands of medium molecular weight and others of low molecular weight; type 2, two or more bands of medium molecular weight; type 3, only one band of medium molecular weight; type 4, without any band. There was a significant predominance of blotting type 1 in group A and type 4 in group C.

Conclusion: These data suggest that IgE immunoblotting is the most useful approach to *A. simplex* allergy diagnosis. (J Allergy Clin Immunol 1997;99:497-501.)

Key words: *Anisakis simplex*, parasite, fish, allergy, IgE immunoblotting, IgE, urticaria, anaphylaxis

Anisakis simplex (Ascaridoidea) is a fish parasite that is responsible for well-known human infection (anisakiasis or anisakidosis).¹ Although helminth parasites induce a dramatic expansion of the T_{H2} lymphocyte subset with

Abbreviations used

MW: Molecular weight

SPTs: Skin prick tests

intense IgE response, allergic reactions are rarely observed in infected individuals.² However, *A. simplex* can induce IgE-mediated reactions with several clinical manifestations ranging from urticaria or angioedema to anaphylaxis.³⁻⁵ The involvement of an IgE-mediated response to *A. simplex* has been proven by means of skin prick tests (SPTs), serum-specific IgE detection, and histamine release in whole blood in some significant cases.³⁻⁶

Recently, we have described the clinical manifestations of 28 patients affected by *A. simplex* allergy. Some of these patients had severe symptoms: 14 had to be treated in an emergency department, and three had to be hospitalized (two had severe angioedema and another had respiratory arrest).⁶

When SPTs and determination of specific IgE to *A. simplex* were performed with standard methods in our department, many positive results were observed in patients without true allergy to *A. simplex* and even in control subjects (healthy donors of the blood bank of our hospital). The apparent lack of specificity of these tests may be explained by a high number of sensitized patients in our area, which could be due to the high fish consumption in our country⁷ and the frequent infection of fish by *A. simplex*.⁸ The low specificity of both diagnostic methods could also be explained by a cross-reactivity with other nematodes and antigens.⁹⁻¹¹

Because IgE immunoblotting with a characteristic pattern has been described as a specific test with which to differentiate anisakiasis from *A. simplex* sensitization,¹² the aim of this study is to assess the usefulness of this test in the diagnosis of allergy to *A. simplex*.

METHODS

Patients

Sixty-one patients, 39 women and 22 men, with a mean age of 47 years (range, 21 to 72 years) were selected. All patients demonstrated a high level of serum-specific IgE to *A. simplex* measured by the CAP system (Pharmacia) and acute symptoms

From ^athe Department of Allergy and Immunology, Santiago Apostol Hospital, Vitoria-Gasteiz, Basque Country; and ^bthe Department of Immunology, Health Institute Carlos III, Madrid.

Supported in part by grant no. 96/0023 of the Fondo de Investigación Sanitaria, a grant of the Foundation of Spanish Society of Allergy and Clinical Immunology, and a grant of the Departamento de Sanidad del Gobierno Vasco.

Received for publication May 14, 1996; revised Oct. 16, 1996; accepted for publication Oct. 31, 1996.

Reprint requests: Luis Fernández de Corres, MD, PhD, Servicio de Alergología, Hospital Santiago Apóstol, C/ Olaguibel, 29 E-01004, Vitoria-Gasteiz, Spain.

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/1/79226