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Pollinosis to *Ricinus communis* (castor bean): an aerobiological, clinical and immunochemical study

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Summary

Background *Ricinus communis* (castor bean) is a species included into the Euphorbiaceae family, common to all the warm regions of the world. Although the allergenicity of its seed is well known, references are scarce regarding the role played by its pollen as a pneumo-allergen.
Objectives To carry out an aerobiological study of this pollen in the Málaga area (southern Spain); describe the physicochemical characteristics of its most relevant allergens; and to demonstrate the existence of patients with respiratory allergy due to this pollen.
Methods A Burkard spore trap was used for the aerobiological study from 1992 to 1996. Skin prick tests with castor bean pollen extract were performed to 1946 patients with rhinitis and/or asthma. Specific IgE levels were measured in castor bean-positive SPT patient sera. Immunochemical characterization of the most relevant allergens was performed using electrophoretic techniques. *In vitro* cross-reactivity studies using positive patient sera were carried out. Nasal challenge tests were done in 32 subjects randomly selected from the sensitized patient group.
Results Castor bean is a perennial pollen with total annual pollen levels never exceeding 1%. One hundred and eighteen (7.7%) patients showed positive prick test (74 rhinitis, 36 rhinitis and asthma, eight asthma). Nine were monosensitized. Specific IgE levels were ≥ 0.35 PRU/mL in 39 (33%) of patient sera. Nasal challenge test: 10 subjects presented non-specific nasal hyperactivity, 15 were positive and seven negative. The molecular masses and isoelectric points of the main IgE-binding proteins, ranged from $\approx 67–15.5/14.5$ kDa and $\approx 4.5–5.5$, respectively. Profilin of the extract was purified by poli-L-proline-Sepharose chromatography and it appeared as one of the most frequent allergens.
Conclusion Castor bean pollen is an allergen which causes respiratory (mainly nasal) symptoms.

Keywords: asthma, castor bean, cross-reactivity, nasal challenge test, pollen, profilin, rhinitis, *Ricinus communis*

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Introduction

The castor bean plant (*Ricinus communis*) originated in Ethiopia and gradually dispersed towards South Africa, the Mediterranean region and warm areas of Asia, until finally establishing itself as a natural species in the majority of warm climate regions of the world.

This plant belongs to the Euphorbiaceae family, and is a very large, rapidly growing, bush (reaching heights of up to 5 m), with a woody, hollow stem. Its leaves are large, palmate, lobulated with a waxy powdery texture and

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Fig. 1. *Ricinus communis* (castor bean) plant.

sometimes of a dark purple colour (Fig. 1). The pollen is basically anemophilous, given the explosive dehiscent nature of the anthers. It is trizonocolporate, isopolar, radiosymmetric and medium sized with a perforated surface (Fig. 2). This species has a very long flowering period, and therefore pollen can be found during any season of the year.

In Málaga, a province situated in the south of Spain on the Mediterranean coast (latitude 36°37′N, longitude 4°19′W), boasting a warm climate the whole year round, the castor bean plant can be found widely distributed, even though its growth is not humanly controlled. It behaves like a rural nitrophilous plant and is found along roadsides, riverbanks, close to gardens and work sites, dumps, etc. It is farmed in different countries world-wide [1,2].

Nowadays modern industry uses the oil extracted from its seeds in the manufacture of explosives, varnishes, lubricants, dyes, plastics, fertilizers, leather, candles, bitumen and cosmetics, as well as laxatives, antifungual and antiparasitic preparations [3,4]. The disease was described in laboratory workers, but has also been noted in employees working in oil processing mills, fertilizer retailers, the upholstery industry and other industrial fields. Other medical problems such as conjunctivitis, rhinitis and urticaria have also been related with this kind of seed and it was, as well as castor bean leaves, described as a producer of atopic dermatitis [5]. Some studies have been carried out to describe the biochemical characteristics of its principal antigens and allergens [6,7].

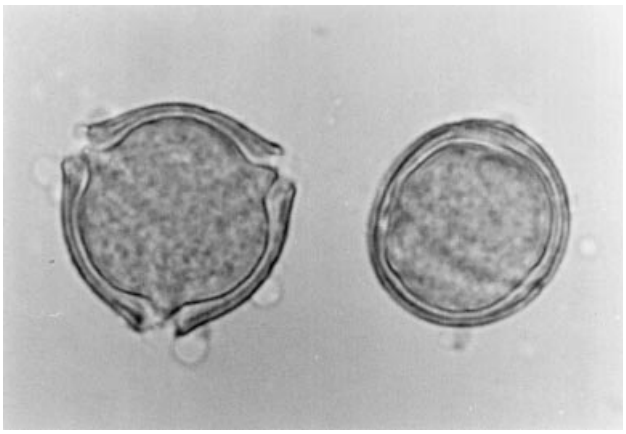

Fig. 2. Castor bean pollen grain (optical microscope).

However, apart from a few studies [8,9] where the allergenic character of its pollen is discussed, little effort has been made to discover the physicochemical properties of its principal pollen allergens. In fact only two studies exist [6,7], and both mainly studying its cross-reactivity capacity.

The aims of this work were to determine the concentrations of castor bean pollen in the atmosphere of the Málaga area (Spain), to study the prevalence of sensitization among patients suffering from allergic rhinitis and asthma, to describe the physicochemical characteristics of its main allergens and its cross-reactivity with other pollens and to prove the existence of patients with allergic rhinitis caused by this pollen.

Materials and methods

Aerobiological sampling

Aerobiological sampling was carried out between 1992 and 1996 using a Burkard Volumetric Pollen Trap [10], situated on the terrace of the Faculty of Medicine of the University of Málaga, 15 m above the ground. This building is located 1 km from the city centre, in an open area, free from obstacles which could deter free air flow. White petrolatum, evenly extended over a Melinex tape, was used as adhesive material. The counts were read by the same observer from four longitudinal bands for each preparation, using a 40× objective (0.45 mm of microscopic field). The hourly determinations were carried out with the help of a small ruler printed on acetate paper, attached to the back part of the slide. The pollinic data was expressed in number of grains of pollen per cubic metre of air per day. The hourly values were presented in percentages of the daily total.

Subjects

1946 consecutive patients from the outpatient Allergy Section (Complejo Hospitalario Carlos Haya, Málaga), diagnosed with allergic rhinitis and/or asthma, were studied between July 1995 and April 1996. Sixty subjects with neither a personal nor family history of atopy, and with negative prick tests to the habitual pneumoallergens were

enrolled in the control group to dismiss false positive castor bean prick test.

Skin tests

Skin prick tests were performed using standardized lancets (Dome Hollister, Leverkusen, Germany), in accordance with the recommendations of the European Academy of Allergology and Clinical Immunology [11]. Prior to the test, all patients suspended treatment with systemic corticosteroids 4 weeks beforehand, topical corticosteroids 2 weeks, antihistamines 10 days and astemizole, 3 months.

We used the most common allergens in our area, including pollen, mites, moulds, danders, and castor bean extract (10 mg/mL). Histamine chlorohydrate at 10 mg/mL and phenolated glycerol saline solution were used as positive and negative controls, respectively.

The pollen extract was diluted in phenolated and glycerinated saline solution at concentrations of: 0.1, 1.0, 10.0 and 50.0 mg/mL. The mean weal areas produced by each concentration was plotted in function of the allergen concentration in a log–log system, and linear regression was carried out. The allergen concentration which elicited a weal equal to that produced by histamine (10 mg/mL), was denominated 1 histamine-equivalent prick (HEP) [12]. One HEP unit was found to be equivalent to 6.2 mg/mL of allergen extract.

Castor bean pollen extract and biochemical measures

Defatted pollen was extracted by magnetic stirring in phosphate buffer. The extract was clarified by centrifugation, filtered through 0.45-μm pore diameter membranes and dialysed by ultrafiltration.

Protein concentration was estimated according to the method of Bradford [13]. Carbohydrate content was measured by both the anthrone method [14] and the orcinol method [15] to determine hexoses and pentoses, respectively.

RAST

Solid-phase antigen was obtained by coupling the extract solution (10 mg/mL) to the 6-mm diameter CNB-activated paper discs as described by Ceska and Lunqvist [16]. RAST was performed (Phadezym RAST, Pharmacia Diagnostics AB, Uppsala, Sweden), in accordance with the manufacturer's instructions

Analytical methods

SDS-PAGE was carried out according to the method of Laemmli [17]. Isoelectric focusing was performed on Isogel agarose plates (FMC Bio Products, Rockland, ME, USA), upon the pH range of 3–10. The proteins were electrophoretically transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Milford, MA, USA), essentially as described by Towbin *et al*. [18]. Immunochemical staining was performed as previously described [19]. When molecular masses and the weight percentages of each protein band in the sample was calculated a densitometer Image Analysis (BioImage System, Millipore) was used.

Glycoproteins were detected after SDS-PAGE and electrotransferred to polyvinylidene difluoride (PVDF) membranes, by using a glycan detection kit (DIG Boehringer Mannheim GmbH, Mannheim, Germany) following the manufacturers instructions.

Chromatographic purification of profilin

Profilin purification was accomplished by affinity purification in a poly-L-proline (PLP) CNBr-activated Sepharose column, as described by Lindberg *et al*. [20].

RAST inhibition

RAST inhibition was carried out according to Yman *et al.* [21]. A serum pool (class ≥ 1) from patients sensitive to castor bean pollen and a serial dilution (0.001–10 mg/mL) of all the inhibitor extracts were used to carried out the assay. The Ag_{50} value is defined as the concentration (mg/mL) of the inhibitor extract which produced a 50% inhibition in the assay.

Nasal challenge test

A nasal challenge test was performed on 32 patients selected at random amongst those with a positive prick test to castor bean and with clinical symptoms of rhinitis, and on 10 subjects from the control group. The study was carried out in the month of October, a low pollination period of this pollen. The patients had not taken any medication during the last month nor had received immunotherapy. Prior to the study, functional respiratory tests were made following the American Thoracic Society Guidelines [22], and a nasal exploration ruled out any mechanical obstruction of the nostrils.

The patients were studied using the active anterior rhinomanometry technique, following the Committee Report on Standardization of Rhinomanometry [23] criteria, by means of a Rhinospir 164 rhinomanometer (Sibelmed, Barcelona, Spain). After spraying 0.2 mL of diluent (human albumin solution, 0.03%), increasing concentrations (0.01, 0.1 and 1 mg/mL) of allergen were sprayed into the same nostril every 15 min until a positive result was achieved.

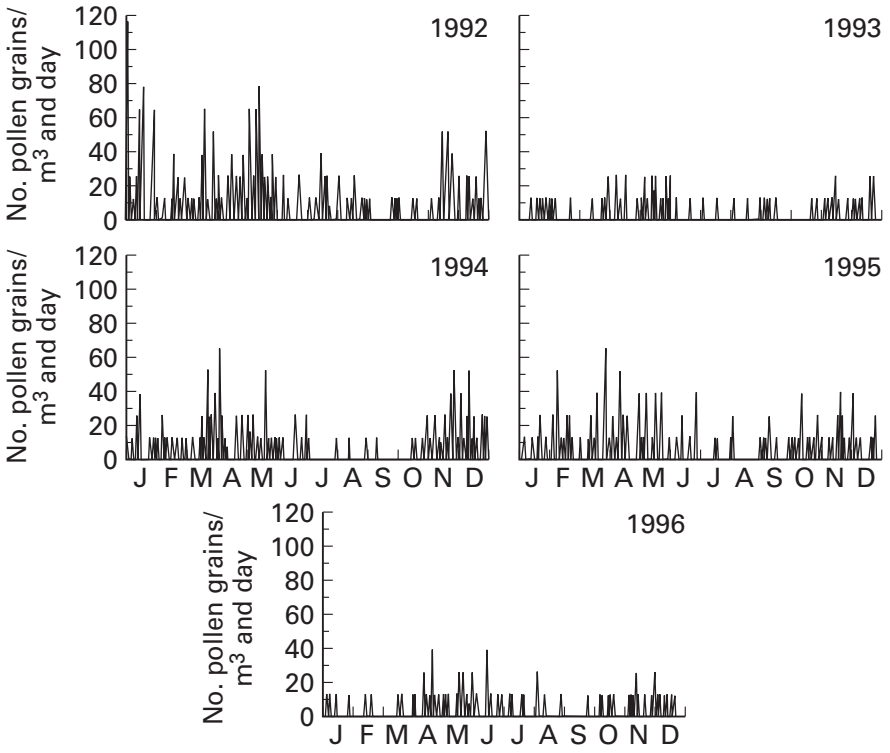

Fig. 3. Daily pollen concentration of *Ricinus communis* registered in the atmosphere of Málaga (Spain) during 1992–96.

Statistical analysis

Statgraphics Plus ver. 3.0 software was used for the statistical analysis, and the statistical significance on the associations was examined by the chi-square test.

Results

Aerobiology

Castor bean pollen was detected in the atmosphere of Málaga the whole year round (Fig. 3), at generally low concentrations, which never exceeded 1% of the total annual pollen count detected going from 1037 grains/m^3 of air in 1993 up to 3357 in 1992. Generally, the greatest concentrations were obtained during the period of November to May, decreasing during the summer, probably due to the hydric stress suffered by the plant during the dry period (Fig. 3). The maximum value detected corresponded with 1st January 1992, with a daily total of 117 grains of pollen per cubic metre of air. With respect to the intradaily variation, the greatest concentrations were registered between 12.00 and 16.00 h, when the increase in the temperature favoured the dehiscence of the anthers. Once the intradaily distribution index had been calculated, the mean value (± SD) of the 5 years sampled was 0.21 (± 0.05) [24] (Fig. 4).

Clinical study

Of the 1946 patients studied, mean age (± SD): 31.2 (± 7.1), 1528 out of them (78.5%, CI: 76.5–80.2) showed one or more positive SPT, with 118 (7.7%, CI: 6.6–8.9), 60 females and 58 males, having castor bean-positive skin tests. Nine of them were monosensitized. The mean age found in the 118 castor bean-positive patients was 29.3

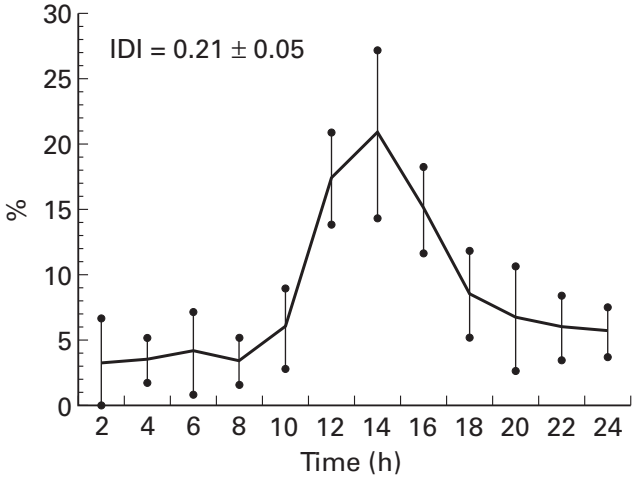

Fig. 4. Mean intradiurnal fluctuations of *Ricinus communis* pollen in Málaga during 1992–96.

Table 1. Clinical characteristics of the selected patients

	Atopics		Nonatopics	
	RC-positive*	*RC*-negative†	SPT negative	Total
Rhinitis	74 (62.7%)	782 (55.5%)	311 (74.4%)	1167
Rhinitis and asthma	36 (30.5%)	587 (41.6%)	78 (18.7%)	701
Asthma	8 (6.8%)	41 (2.9%)	29 (6.9%)	78
Total	118	1410	418	1946

**Ricinus communis*-SPT-positive patients. †Patients SPT-positive to pneumoallergens other than castor bean.

(± 6.7) years. The pollen and histamine mean weal area was 59.4 (± 49.4) mm^2 and 36.2 (± 12.3) mm^2, respectively. Four hundred and eighteen patients and 60 control subjects had negative SPT. Clinical characteristics of the studied patients can be seen in Table 1.

When comparing the subgroups of diseases in the atopic groups (castor bean positive and negative), we found a *P*-value of 0.009 ($\alpha = 0.05$) in the rhinitis and asthma patients. We did not find differences in the other groups.

Amongst the 118 sensitized patients, 110 (93.2%, CI: 87.0–97.0) suffered from rhinitis and 44 (37.2%, CI: 28.4–46.6) from asthma, distributed in the following way: 54 (45.8%, CI: 36.3–55.6) perennial rhinitis, 23 (19.5%, CI: 12.8–27.8) perennial rhinitis and asthma, eight (6.8%, CI: 5.9–7.6) perennial asthma, 20 (16.9%, CI: 10.6–24.9) seasonal rhinitis and 13 (11%, CI: 9.9–18.0) seasonal rhinitis and asthma.

Specific IgE measurements

The specific IgE against castor bean found in the sera of the 118 patients with positive SPT, was measured by means of the RAST technique. Of these, 39 (33%, CI: 20.0–36.9) were positive, where 16 were class 1, 16 class 2, 6 class 3 and 1 class 4.

Biochemical characterization of the extract

The protein and carbohydrate content of the extract was calculated, obtaining 45% (w/w) protein content, 10% (w/w) hexose content and 9% (w/w) pentose content.

The protein composition of the pollen extract was studied using SDS-PAGE and IEF, showing a protein pattern of 22 bands with molecular masses ranging between ≈ 88 and 13 kDa. The molecular masses of the most predominant of these were ≈ 88, 57, 42, 39, 37, 27, 16 and 15 kDa, and their weight percentages were 5.2, 9.3, 6.3, 9.1, 17.4, 8.6, 10 and 9.6%, respectively (Fig. 5a). IEF revealed proteins bands ranging from 6.8 to 4.0. The pI of the most abundant were 5.1, 5.0, 4.9, 4.8, 4.6, 4.4, 4.3, and 4.0, and their weight percentages were 11, 12, 14, 8, 14, 12, 10 and 7%, respectively (Fig. 6).

SDS-PAGE was used to study the profilin of the extract, purified by PLP-Sepharose chromatography, and two bands

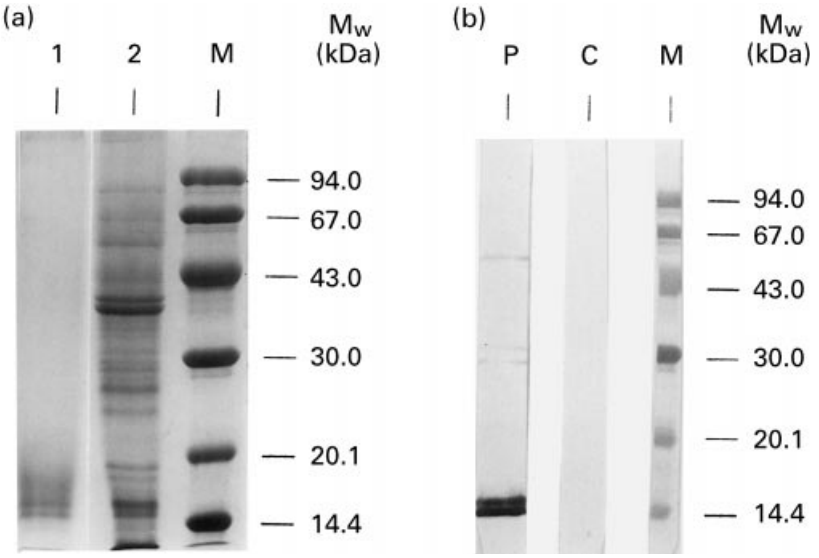

Fig. 5. (a) SDS-PAGE electrophoretic results. Lane 1: *Ricinus communis* purified profilin. Lane 2: *R. communis* pollen extract. Lane M: molecular mass marker. (b) SDS-PAGE Immunoblotting results. *R. communis* pollen incubated with rabbit antisunflower profilin serum. Lane P: immunoblotting incubated with antiprofilin rabbit serum. Lane C: immunoblotting incubated with control serum (nonimmunized rabbit serum). Lane M: molecular mass marker.

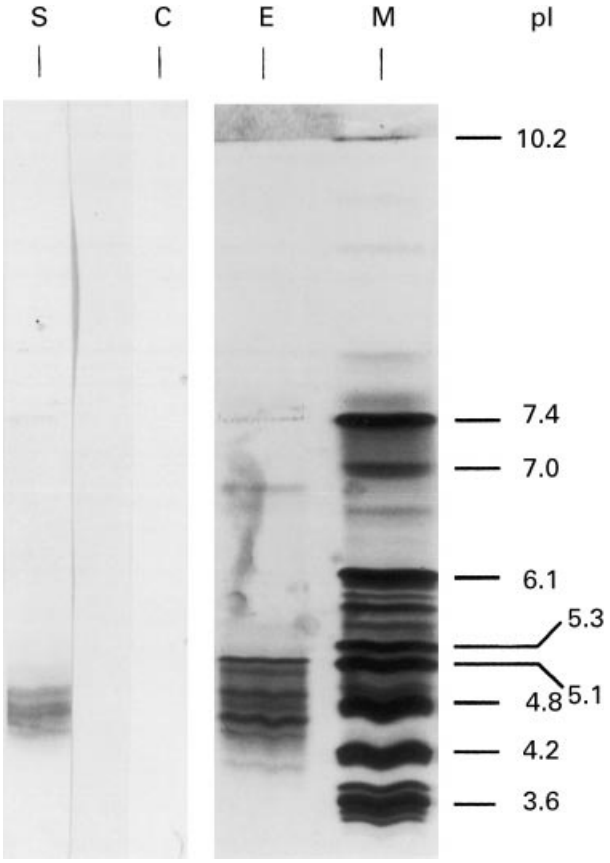

Fig. 6. *Ricinus communis* pollen extract IEF after Coomassie Brilliant Blue R-250 staining and IEF immunoblotting. Lane S: immunoblotting incubated with a pool of patient sera. Lane C: immunoblotting incubated with control serum (pool from nonatopic subjects' sera). Lane E: castor bean pollen extract IEF after staining. Lane M: pI marker.

of 15.3 and 14.6 kDa appeared (Fig. 5a). The weight percentages of each were 41 and 59%, respectively. Glycoprotein detection showed the non-glycosylated nature of profilin, as occurs in other cases [25].

Immunochemical characterization of the extract

The electrophoresed and electrotransfered extract was incubated with patient sera (class ≥ 1) showing 13 relevant IgE-binding bands with the following apparent molecular masses: 67, 53, 45, 36.5, 34.5, 31.5, 27, 25, 23, 19, 17, 15.5/14.5 kDa (Fig. 7). The bands revealed with the control serum also appeared when the membrane was incubated with a pool of sera of allergic patients non-sensitized to castor bean pollen (data not shown), and must be an non-specific binding. When it was revealed using antisunflower profilin rabbit serum, two IgE-binding bands of 15/14.5 kDa appeared (Fig. 5b), revealing the profilin identity of the 14.5/15.5 kDa doublet seen in the immunoblotting extract in a large number of patients (Fig. 7).

IEF immunoblotting of the extract, was incubated with a serum pool of patient sera with castor bean-IgE levels ≥ 0.35 PRU/mL, produced relevant IgE-binding bands ranging between ≈ 4.5–5.5 and the principal bands showed pI values of 5, 4.9, 4.85, 4.80, 4.5 (Fig. 6).

Cross-reactivity studies

Cross-reactivity studies were carried out with RAST inhibition assays. When the castor bean extract was used as inhibitor, an $Ag_{50} = 7.2\ \mu g$/mL and 100% inhibition at 1 mg/mL was obtained, whereas when other extracts were used the following values of Ag_{50} and percentage inhibition at 1 mg/mL were obtained: *Betula verrucosa* $Ag_{50} = 70\ \mu g$/mL and 65% inhibition; *Olea europaea* $Ag_{50} = 71\ \mu g$/mL and 63% inhibition; *Helianthus annuus* $Ag_{50} = 110\ \mu g$/mL and 62% inhibition; *Zygophyllum fabago* $Ag_{50} = 120\ \mu g$/mL and 60% inhibition; *Parietaria judaica* $Ag_{50} = 154\ \mu g$/mL and 60% inhibition; *Lolium perenne* $Ag_{50} = 260\ \mu g$/mL and 62% inhibition; *Mercurialis annua* $Ag_{50} = 290\ \mu g$/mL and 60% inhibition. The results obtained in the assays are shown in Fig. 8.

Nasal challenge test

The mean age ($\pm$ SD) of the 32 patients (20 male and 12 female), randomly selected from the group of 118 patients with positive skin test, was 30.1 ($\pm$ 10.5) years. These patients suffered from rhinitis (26 perennial and six seasonal), and 15 (46.8%, CI: 29–65.1) also suffered from asthma (12 perennial and three seasonal). Sensitization to the other allergens tested can also be observed in Table 2. Four patients were monosensitized.

The size of the papules yielded by the castor bean and histamine was 54.1 ($\pm$ 33) and 36.6 ($\pm$ 12.9) mm^2, respectively. This can be seen in Table 3, together with the RAST and nasal challenge test results of these 32 patients. Thirteen patients (40.6%, CI: 23.6–59.3) had a positive RAST (five class 1, seven class 2, and one class 3). In accordance with the criteria used [23], 10 subjects presented non-specific nasal hyperreactivity (a decrease of $\geq 25\%$ in the baseline flow after inhalation of the solution of the negative control). Of the remaining 22 subjects, 15 (68.2%, CI: 45.1–86.1) were positive and seven (31.8%, 13.9–54.6) negative. Only two patients showed late responses. Three out of the four monosensitized patients showed positive nasal challenge test and one was discarded (nasal hyperreactivity). In the majority of the subjects, symptoms were reproduced (mainly pruritus and rhinorrhea) at a concentration of 1 mg/mL of inhaled allergen, although three patients reacted

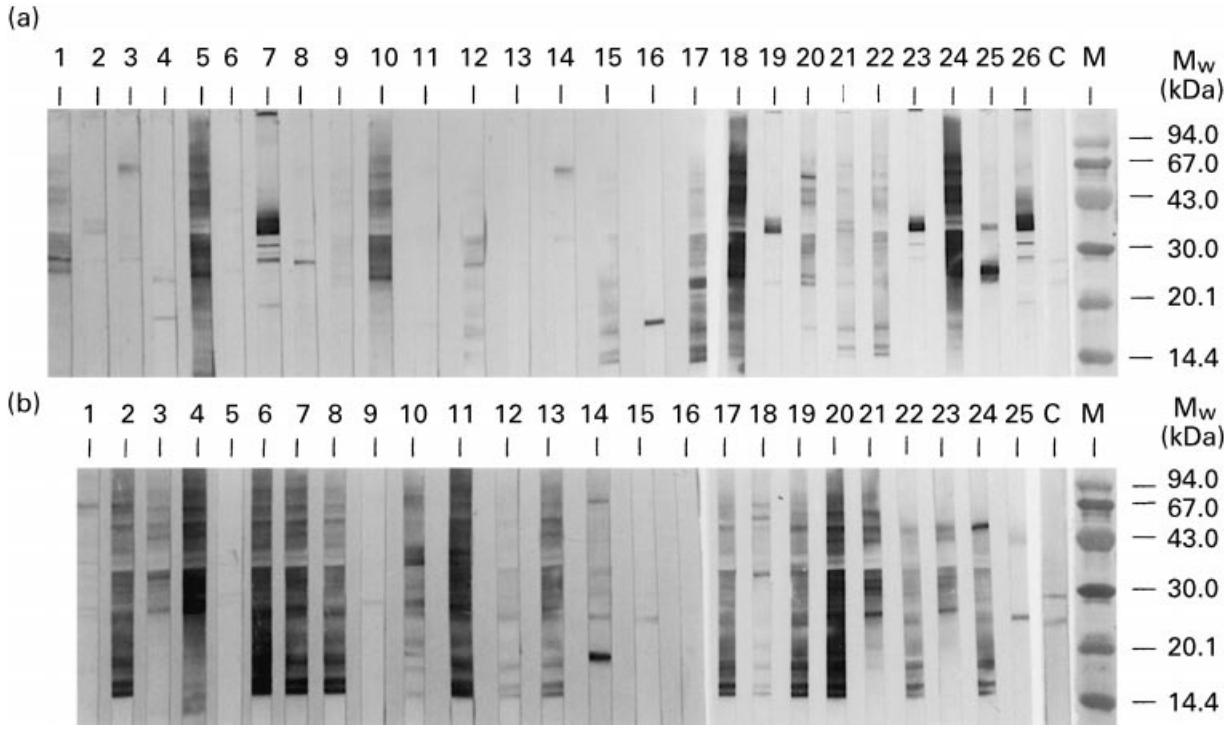

Fig. 7. SDS-PAGE immunoblotting. (a) and (b) *Ricinus communis* pollen extract incubated with selected individual sera (class ≥ 1). Lane 1–26: patient sera. Lane C: control serum (pool from nonatopic subjects' sera). Lane M: molecular mass marker.

to the lowest allergen concentration (0.01 mg/mL). Nasal challenge in the 10 control subjects was negative.

Discussion

The castor bean plant is widely extended in the warm regions of the world and its anemophilous character and long-flowering period increase the interest of this pollen from an allergological point of view.

The aerobiology of this pollen is not, however, sufficiently known, although it has been studied in some countries using different methodology. Pollen can be found in every season of the year [4,26,27].

During the 5-year sampling period of our work, the amount of pollen detected in the atmosphere of Málaga was low and distributed throughout the whole year, with maximum concentrations seen between November and May (Fig. 3). With respect to the intradaily distribution, this type of pollen behaved in a fairly stable manner during the studied years, with a maximum pollination peak at around 14.00 h (Fig. 4), given that high midday temperatures provoke massive dehiscence of the anthers and thereby, the subsequent liberation of pollen into the atmosphere. This data could be of interest for the establishment of prophylactic measures for patients.

Very few bibliographical references exist regarding the allergenicity of castor bean pollen. In 1966, Lindebaum [8] published in Israel a case of seasonal rhinitis and asthma monosensitized to this pollen.

Some studies in India find different percentages (from 9 to 43%), possibly due to different patient criterion selection and skin testing techniques (intradermal, scratch) [26,28]. Singh *et al.* [29] found 22 positive skin tests in a study of the biological standardization of castor bean and *Holoptea integrifolia* pollens, providing no clinical or epidemiological data of the population tested.

In our study, 118 subjects showed positive skin tests to castor bean pollen, a lower percentage of sensitization (7.72%) than in those papers previously referred to, which can be due to the use of a more rigorous methodology in the selection of patients and the realization of skin tests, or to a lower sensitized population as a consequence of a lower atmospheric concentration of this pollen, a fact which cannot be contrasted with previous literature.

In the statistical analysis we observe that the sensitization to castor bean makes a difference in the distribution of the subgroups: we found a smaller number of castor bean-positive patients suffering both rhinitis and asthma as compared with the castor bean-negative atopic group. We did not find any other statistical significance when comparing the other subgroups; however, we can observe a tendency to a higher incidence of rhinitis. This latter point

Table 2. Characteristics of 32 patients sensitized to castor bean

Patient no.	Sex	Age (years)	Symptoms*	Other sensitizations†
1	F	19	R	Cat
2	F	33	R, A	Che, Mer, mites
3	M	30	R	Grass, Pla, Ole, Che, Plan
4	F	36	R, A	Grass, Pla, Mer, cat, Plan
5	M	40	R	Che, Ole
6	F	47	R	Grass, Pla, Ole, Che, Plan, Art, Par, Mer
7	F	32	R, A	Ole, Plan, Mer
8	M	18	R, A	Grass, Ole, mites, Mer, cat
9	M	17	R, A	Art, mites
10	M	35	R	Grass, Pla, Ole, Che, Art, Par, Mer
11	M	18	R	Grass, Ole, cat
12	F	57	R	–
13	M	18	R, A	Ole, Plan, Alt
14	M	21	R	Par, Mer
15	M	37	R, A	Pla, Ole, Art, Par, Mer, mites
16	M	31	R, A	Grass, Pla, Ole, Plan, Par, Mer, Alt, cat
17	M	24	R	Grass, Pla, Ole, Che, Plan, Art, Mer, mites
18	M	36	R	Ole, Che, Mer
19	F	48	R, A	–
20	F	25	R	Grass, Pla, Ole, Che, Plan, cat
21	M	32	R	Grass, Pla
22	M	30	R	–
23	M	22	R, A	Grass, Ole, dog, cat
24	M	38	R, A	Grass, Ole, Par
25	F	17	R	Pla, Art
26	F	38	R, A	Grass, Ole, Plan, Che, Mer, cat
27	M	19	R	Grass, Ole, Che, Mer, mites, cat
28	M	18	R, A	Grass, Ole, Che, Mer, dog, cat
29	F	41	R	Che
30	M	18	R, A	Plan, Mer
31	M	34	R, A	Grass, Ole, Che, Plan
32	F	35	R	–

*R: rhinitis A: asthma. †By prick test. Ole: *Olea*. Che: *Chenopodium*. Plan: *Plantago*. Pla: *Platanus*. Mer: *Mercurialis* Art: *Artemisia*. Par: *Parietaria* Alt: *Alternaria*. Cat: cat dander. Dog: dog dander. Mites: dust mites

might be confirmed increasing the size of the sample and also studying a higher number of monosensitized patients; currently we are working on this.

In 1992, Singh *et al*. [2] studied the intraspecific protein variation of the castor bean pollen extract, with respect to different circumstances, in all cases, as in ours, the acidic proteins with a pI of between 4.0 and 5.5, were the principal ones, with minor changes in the basic proteins. The same study, using SDS-PAGE, described a slightly higher variance in the molecular masses of the allergens, in connection with different circumstances and growth stages, and highlighted two bands of 66 and 70 kDa as the principal proteins of the extract in terms of weight. However, and possibly due to geographical differences, the most relevant proteins found in our extract corresponded with molecular masses of 57, 39, 37, 16 and 15 kDa.

The cross-reactivity between allergens from the castor bean seed and other sources (different parts of the same plant, such as leaves and pollen, and other seeds; coffee bean) has been studied, with diverse results being obtained [4,6,30]. Since 1963, cross-reactivity between the seed and the pollen allergens has indeed been reported (RAST-inhibition, SDS-PAGE immunoblotting inhibition), showing differences in sensitivity to pollen amongst groups of castor bean-sensitive patients from different studies. Recently Singh *et al.* [6] described the molecular masses

Table 3. RAST, SPT and nasal challenge test in 32 patients sensitized to *Ricinus communis*

Patient no.	Prick test		RAST		Nasal challenge test	
	Allergen weal*	Histamine weal*	PRU/mL	Class	Immediate response† (mg/mL)	Late response
1	27.24	24.7	0	0	NH	Neg
2	32.13	45	0.66	1	NH	Neg
3	37	19.2	0	0	Neg	Neg
4	25.82	76.1	4.67	3	0.02	Neg
5	26.3	37.3	0	0	Neg	Neg
6	25.24	93.2	0	0	NH	Neg
7	63.54	24.2	0	0	Neg	Neg
8	6.52	41.30	3.11	2	NH	Neg
9	70	64.2	0	0	Neg	Neg
10	33.4	58	0	0	0.02	Neg
11	36.4	29.5	0.49	1	Neg	Neg
12	31.34	129.4	0.84	2	2	Neg
13	20.56	34.2	0	0	NH	Neg
14	44.18	29.3	0	0	0.02	Neg
15	30.5	54.7	3.29	2	NH	Neg
16	36	56.3	0.38	1	0.02	Neg
17	32	54.4	1.56	2	0.02	Neg
18	38.87	67.3	0	0	0.2	Neg
19	44.34	34.0	0	0	NH	Neg
20	37.45	69.4	0.89	2	NH	Neg
21	55.59	41.3	0	0	Neg	Neg
22	43.18	25	0.4	1	0.02	Neg
23	35.24	54.7	0	0	0.2	Neg
24	41.1	28	0	0	0.02	Neg
25	56.80	38.4	0	0	0.02	Neg
26	38.1	45.5	0	0	0.02	Pos
27	49.86	90	0.42	1	2	Neg
28	26.1	69.4	3.16	2	Neg	Neg
29	26	18.3	0	0	0.02	Neg
30	24.24	6.7	0	0	NH	Neg
31	43	98.4	1	2	NH	Neg
32	33	73	0	0	0.02	Pos
Mean value	54.1 ± 33	36.6 ± 12.9				

*Allergen and histamine weal area (mm^2). †Allergen concentration inducing positivity of challenge test. NH: non-specific nasal hyperreactivity. Neg: negative. Pos: positive.

of the principal bands appearing in the pollen extract under SDS-PAGE immunoblotting, revealed with sera from castor bean seed-allergic patients, as being of 52, 48, 39.5, 33.5, 19 and 14.5 kDa, and these are fairly similar to those found in our study with sera from patients allergic to castor bean pollen. Cross-reactivity studies are interesting, in order to try out specific immunotherapy with a castor bean pollen extract (free from the problematic presence of a toxin protein as ricin) upon patients allergic to the castor bean seed, which is a well-recognized and frequent occupational allergy. Until now, this kind of therapy has been used to treat a pollinic patient, with very promising results [8]. Although the biological activity value of our extract is close to that referred to by Singh *et al.* [2] it is very important to obtain a local, standardized castor bean pollen extract.

The pattern of IgE-binding bands, using sera of SPT positive and low level of specific IgE patients, is similar although less intense (data not shown) to the one obtained using sera with class ≥ 1, reflecting the presence in sera of low affinity IgE-specific antibodies, low levels of IgE antibodies or reflecting low weight percentages of the

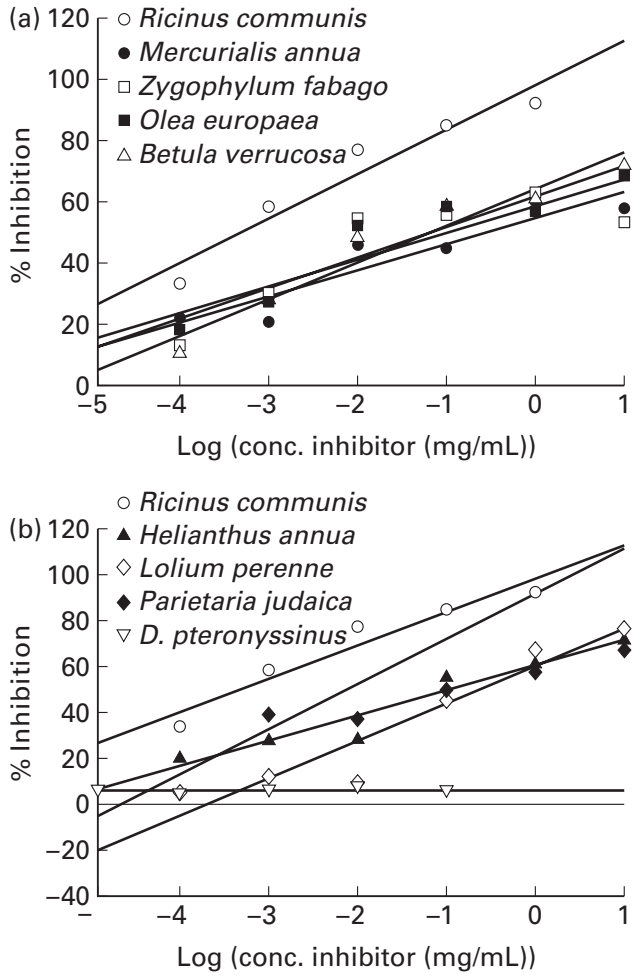

Fig. 8. RAST-inhibition results. *Ricinus communis* used as solid phase and the different pollen extracts in study as free phase.

relevant allergens in the extract. This could explain the low RAST values obtained in the patient sera (67% of patients with class = 0). RAST inhibition showed a 60–70% inhibition value with heterologous extract and this could be due to the presence of a widely distributed pollen allergen, such as profilin.

Of the 32 patients upon which nasal challenge was carried out, 13 had a positive RAST and 26 suffered from perennial rhinitis. The large number of subjects with non-specific nasal hyperreactivity is very surprising and could be related to the coefficient of variation of the test, ranging from 9 to 14% in normal subjects and up to 30.6% in rhinitic sufferers [31,32]. However, we could guess other possible interpretations such as the sensitization to mites seen in three patients; a sub-clinical irritation of the nasal mucosa produced by the same amounts of pollen present in the atmosphere during the whole year; or the levels of this pollen present at heights lower than 15 m; and also the areas close to the bush could experience earlier and greater levels, as has been described for other pollens [33]. In fact, patients number 12 and 29, who live in a rural area with a large amount of castor bean plants near to their houses, presented clear nasal hyperreactivity that disappeared 1 week after having been absent from their home. Of the remaining 22 subjects, 15 presented a positive nasal challenge test (the majority at a concentration of 1 mg/mL). We note that one out of the four monosensitized patients showed nasal hyperreactivity and the other three were positive and likewise all of them had perennial symptoms.

Only one study with six patients [34] deals with the ability of the castor bean pollen to induce bronchial asthma. As we found 37% of our patients suffering asthma, further studies on this point are currently ongoing.

In summary, castor bean pollen is detected in the atmosphere of Málaga the whole year round, although at low concentrations. In our area, 7.72% of patients with allergic rhinitis and/or asthma have positive skin tests to this pollen, which is capable of provoking rhinitis in allergic patients, as can be seen in the nasal challenge test. Further studies are currently ongoing to study the ability of castor bean pollen to induce bronchial asthma.

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