

Serum reactivity to other indoor ficus plants in patients with allergy to weeping fig (*Ficus benjamina*)

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Ornamental plants have long been used for indoor decoration. An example is the india rubber tree (*Ficus elastica*). With the increased popularity of green plants, both in private homes and public premises, small-leaf species, such as weeping fig or *Ficus benjamina* (Fb), have become widely used. Exposure to dust from Fb may cause sensitization and allergic airway symptoms, which among occupationally exposed plant keepers occur both in atopics and non-atopics. The serum reactivity to sap extracts from Fb and seven other indoor plants of the genus *Ficus* were investigated with RAST and a RAST inhibition technique, using sera from 12 atopic subjects and 12 plant keepers, sensitized to Fb. The allergenic similarity between the different extracts was found to be extensive. The specific IgE antibodies with the highest concentrations in serum were those against Fb and its variegate form, "star light", with decreasing values for the other species, especially those with larger leaves. The binding of IgE antibodies to the other *Ficus* RAST discs could be completely inhibited by extract of Fb. These reactions were probably due to cross-reactivity. Sensitization is believed to occur by inhalation of allergen-enriched dust, emanating from the leaves of the plants. The high allergenic potency of the species with many small leaves may be due to their large total leaf area.

Key words: allergy; cross-reactivity; *Ficus*; *Ficus benjamina*.

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During recent decades green plants have become very popular in interior decoration and *Ficus benjamina* (Fb) or weeping fig is one of the most common non-flowering green plants used for this purpose in Europe.

We have previously reported that hypersensitivity of the immediate type to Fb occurs in about one-fourth of occupationally exposed plant keepers (1, 2). Sensitization resulting from non-occupational exposure, for example in homes or offices, appears to occur mainly in clearly atopic subjects, the occurrence among such exposed patients referred for allergological investigation in the Stockholm area being about 6% (3).

Sensitization probably occurs through inhalation of airborne, allergen-enriched dust emanating from the leaves of the plants. The symptoms

are rhinoconjunctivitis and/or bronchial asthma and sometimes contact urticaria. Since exposure occurs indoors all the year round, the symptoms are more or less perennial.

The genus *Ficus* has over 1000 species and belongs to the mulberry family (*Moraceae*). About 10 species and several varieties are available as indoor plants on the Swedish market. In this study we have investigated the allergenic similarity in extracts from Fb and seven other pot plants of the genus *Ficus*.

SUBJECTS AND METHODS

Patients

Serum samples were chosen from 12 occupationally exposed ("high-exposed") plant keepers

(Nos. 1–12), of whom eight were non-atopics and 12 “low-exposed” atopic patients (Nos. 13–24) sensitized to Fb. Sensitization to Fb was defined as exposure to Fb and positive skin prick test ($\geq 3+$) and RAST (≥ 0.4 PRU/ml). Atopics were defined as having a skin prick test reaction of 3+ or more and/or a RAST score of 2 (≥ 0.7 PRU/ml) or more to at least one common allergen.

All “high-exposed” plant keepers had rhinoconjunctivitis and/or bronchial asthma to which Fb-sensitivity undoubtedly was clinically important. In contrast, since the “low-exposed” atopic patients were sensitized to several common inhalant allergens, the importance of Fb sensitization for the allergic symptoms in this group was sometimes difficult to evaluate. However, in some of the patients (Nos. 13, 16, 18, 20 and 24) the clinical importance was obvious. Five (Nos. 1, 3, 6, 8 and 9) plant keepers were subjected to bronchial provocation tests and the others to rhinoconjunctival provocation tests, all with positive results. The challenge studies have been previously reported in detail (2, 5).

It was not known whether exposure to other Ficus plants than Fb had taken place in the two groups. It could only be assumed that practically all the plant keepers had been exposed, to some extent, to the other seven investigated plants. It was assumed on the basis of the patients’ reports that the atopic patients had had little or negligible exposure to non-Fb Ficus plants, with the possible exception of *Ficus elastica* “robusta” (Fer).

Allergen extracts

Extracts of eight commonly used ficus species were prepared: Fb, *Ficus benjamina* “star light” (Fbs), *Ficus australis* (Fa), *Ficus cyathistipula* (Fc), *Ficus westland* (Fw), *Ficus lyrata* (Fl), *Ficus nitida* (Fn) and Fer. Sap (latex) from the plants was collected drop-wise into a test tube after cutting twigs or scarification of the stem. The sap was extracted overnight in physiological saline 1:10 w/v (for Fb, 0.3% phenol was also added). The allergen extract was centrifuged at $1000 \times g$ for 20 min and the supernatant was filtrated through a 0.22 μ m Milipore® filter. Fa was not used in the inhibition studies, as we ran out of

extract and had difficulties in finding a new plant. One batch of allergen extract was used in all investigations with Fb, Fbs, Fa, Fl, Fc and Fer, while two different batches were used in the studies with Fw and Fn. RAST with the two batches revealed no major differences in allergen composition. The RAST results for the two batches differed by less than 20% when the same sera were tested.

Serum IgE reactions

The content of Fb allergens in the various plant species was evaluated by estimation of correlation coefficients between RAST values obtained after testing with the different extracts and by RAST inhibition.

RAST

RAST was performed according to the manufacturer’s instructions (Pharmacia Diagnostics AB, Uppsala), using the commercially available ^{125} I-labelled anti-IgE (Phadebas RAST); the results are expressed in PRU/ml as derived from the Phadebas Reference System (Pharmacia Diagnostics AB, Uppsala). The eight Ficus discs were prepared by coupling 10 μ l (Fa, Fbs, Fc, Fer and Fw), 2 μ l (Fb) or 1 μ l (Fl and Fn) of allergen extract 1/10 w/v to each CNBr-activated paper disc as previously described (7, 6). The discs were tested against non-atopic sera samples with IgE concentrations of 10 to 4000 kU/l. All discs had a non-specific uptake of < 0.35 PRU/ml. RAST with the various Ficus discs were performed in duplicate.

RAST inhibition

Extracts and sera were diluted in 0.05 M phosphate buffer, pH 7.4, containing 0.15 M saline and 0.02% bovine serum albumin. Serum was diluted to give approximately 5000 counts per minute (cpm) in RAST. Forty microlitres of Ficus extract in 10-fold dilutions were mixed with an equal volume of the appropriate dilution of a serum sample and incubated at 4°C overnight. The concentration of free IgE antibodies was then determined

with RAST as described above. Most of the tests were made in duplicate. The results are expressed in per cent of the cpm value of the positive serum dilution after inhibition with allergen extract diluent, according to the following formula:

$$\text{per cent inhibition} = \frac{\frac{\text{cpm after inhibition with extract}}{\text{cpm after inhibition with diluent}}}{\text{cpm after inhibition with diluent}} \times 100$$

A background value (buffer value) averaging 200 cpm was always subtracted from the actual count rate.

Statistical analysis

To estimate the strength of relationships between the various species of *Ficus* plants, Spearman rank correlation coefficients between

RAST scores (positive to at least one of the two species) were calculated. The geometrical means of the RAST values were used in the two-tailed Student's *t* test to compare differences between negative and positive sera. To estimate the differences between the two groups of occupationally (Nos. 1-12) and non-occupationally (Nos. 13-24) sensitized persons, the Wilcoxon two-sample test was used. For all tests $P < 0.05$ was considered significant.

RESULTS

All the 24 patients with positive RAST to Fb, were also positive to Fbs. In 22 of the 24 patients RAST was positive to Fn, Fw and Fa, in 20 patients to Fl, in 16 to Fc and in five to Fer. The results of the RAST, the total IgE concentrations and the atopic status are shown in Table 1.

Table 1.

Results of RAST (PRU/ml) to eight indoor *Ficus* (F) plants and total IgE concentrations in 12 occupationally exposed (Nos. 1-12) and 12 "low-exposed" atopic (nos. 13-24) patients sensitized to *Ficus benjamina*

Patient No.	Atopy	Fb	Fbs	Fw	Fa	Fn	Fl	Fc	Fer	Total IgE (kU/l)
1	+	23	26	19	14	17	12	4.6	0	430
2	+	28	37	26	36	23	30	44	4.1	970
3	-	100	117	66	63	58	38	7.1	0	400
4	+	15	20	12	13	12	6.3	6.0	0.4	150
5	-	16	18	14	9.9	11	8.6	6.3	0.4	90
6	-	22	20	13	9.3	13	2.7	1.9	0	100
7	-	5.2	4.3	4.2	4.0	4.5	4.5	2.5	0	41
8	-	4.6	7.0	2.8	3.7	2.7	2.9	0	0	54
9	+	177	163	89	102	105	68	13	2.0	2000
10	-	2.2	1.8	1.0	0.9	1.0	0.7	0	0	170
11	-	4.8	4.9	3.4	2.5	2.3	1.3	0.7	0	40
12	-	15	17	10	8.9	8.1	5.7	0.5	0	41
Median		15.5	18.5	12.5	9.6	11.5	6.0	3.6	0	125
13	+	46	53	21	18	16	12	0.6	0	110
14	+	1.1	2.2	1.2	2.0	1.6	0.4	3.2	0	230
15	+	3.4	4.8	2.5	3.6	2.5	2.9	0	0	270
16	+	25	29	15	11	14	9.4	2.3	0.4	970
17	+	0.8	2.8	0.5	0	1.6	0	2.2	0	140
18	+	13	14	4.7	1.7	4.4	0.8	0	0	140
19	+	2.5	2.6	1.1	0.6	0.5	0.5	0	0	360
20	+	12	11	2.8	6.5	2.8	5.0	1.4	0	350
21	+	0.5	0.6	0	0.5	0	0	0	0	87
22	+	1.1	1.0	0	0	0	0	0	0	69
23	+	1.0	1.1	0.4	0.6	0.5	0	0	0	33
24	+	77	75	24	20	27	20	1.8	0	390
Median		3.0	3.8	1.9	1.9	2.1	0.7	0.3	0	185

Fb = *F. benjamina*, Fbs = *F. Benjamina* "star light", Fn = *F. nitida*, Fw = *F. westland*, Fa = *F. australis*, Fl = *F. lyrata*, Fc = *F. cyathistipula*, Fer = *F. elastica* "robusta".

Table 2.

Correlation coefficients between RAST values for eight *Ficus* plants in 24 patients sensitized to *Ficus benjamina* (for abbreviations, see legend to Table 1)

	Fb	Fbs	Fw	Fa	Fn	Fl	Fc	Fer
Fb		0.98 (n = 24)	0.98 (n = 24)	0.95 (n = 24)	0.97 (n = 24)	0.95 (n = 24)	0.63 (n = 24)	0.47 (n = 24)
Fbs	< 0.001		0.98 (n = 24)	0.95 (n = 24)	0.98 (n = 24)	0.93 (n = 24)	0.65 (n = 24)	0.49 (n = 24)
Fw	< 0.001	< 0.001		0.96 (n = 23)	0.98 (n = 22)	0.94 (n = 22)	0.66 (n = 22)	0.51 (n = 22)
Fa	< 0.001	< 0.001	< 0.001		0.96 (n = 23)	0.97 (n = 22)	0.70 (n = 23)	0.53 (n = 22)
Fn	< 0.001	< 0.001	< 0.001	< 0.001		0.94 (n = 22)	0.67 (n = 22)	0.48 (n = 22)
Fl	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		0.59 (n = 21)	0.52 (n = 20)
Fc	< 0.002	< 0.001	< 0.001	< 0.001	< 0.001	< 0.006		0.66 (n = 16)
Fer	< 0.02	< 0.02	< 0.02	< 0.02	< 0.03	< 0.02	< 0.006	

Upper right field shows correlation coefficient (*r*) and number of patients (*n*) with positive RAST to at least one of the two plants. Lower left field shows the degree of significance of the respective correlation.

($P < 0.07$), Fbs ($P < 0.09$) and Fer ($P < 0.14$). There were no differences in total IgE between the groups.

RAST correlation

There was a correlation between the RAST data for the various *Ficus* species, indicating presence of Fb allergens in the investigated species (Table 2).

Since the correlation data presented in Table 2 are based on all observations, the validity for Fer could be questioned, because of the excessive number of zero values. However, calculations based only on the five sera reacting positively to Fer also correlated significantly with Fb ($P < 0.04$), Fbs ($P < 0.03$), Fw ($P < 0.03$), Fa ($P < 0.02$), Fn ($P < 0.03$), Fl ($P < 0.02$) and Fc ($P < 0.002$). Similar, significant correlations were also obtained with the sera positive to Fc and Fl.

Compared with the low-dose, non-occupationally exposed group, the plant keepers had significantly higher RAST scores to Fw ($P < 0.04$), Fa ($P < 0.03$), Fn ($P < 0.05$), Fl ($P < 0.04$) and Fc ($P < 0.03$), but not to Fb

RAST inhibition

A complete chessboard RAST inhibition, where all extracts in serial dilutions were allowed to inhibit a positive RAST to any of the eight allergens, was performed in four patients (Nos. 2 and 3 from the occupational group and Nos. 13 and 24 from the non-occupational group). To simplify the interpretation of the sometimes rather complicated dose-response results, the maximum inhibiting capacities are expressed in four classes, where 1 = 0–24 %, 2 = 25–49 %, 3 = 50–74 % and 4 = 75–100 %.

Fb extract completely inhibited the RAST reactions to all eight *Ficus* discs, with the exception of tests with serum No. 2, where inhibition was 50–74 % to Fw, Fl and Fc discs. The inhibiting capacity of the extracts to the RAST reaction to Fb discs was complete for Fb (the homo-

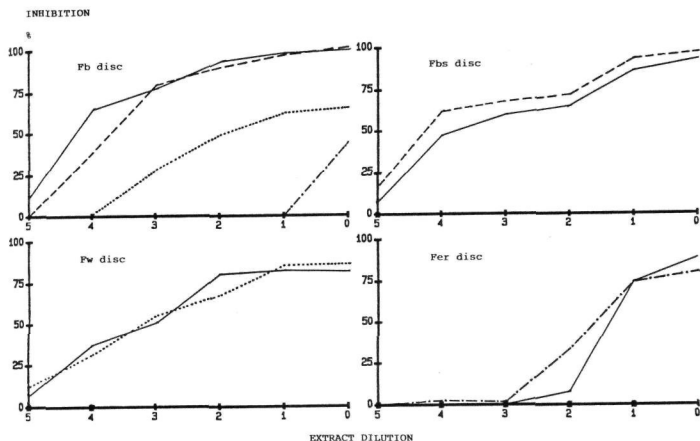


Fig. 1. The inhibitory effect of extracts in 10-fold dilutions (undiluted = 0 to 10^{-5} = 5) of Fb = *Ficus benjamina* ———, Fbs = *Ficus benjamina* "star light" -----, Fw = *Ficus westland* and Fer = *Ficus elastica* "robusta" - - - - - on a positive RAST to discs coated with allergens from Fb, Fbs, Fw and Fer, respectively.

Fer, this reaction is probably associated with the sensitization to Fb, since the Fer-positive sera showed significantly higher titres to the other *Ficus* plants, and, moreover, the IgE levels were higher. Further, this pattern was generally also observed for Fc and Fl.

On comparing the two exposure groups, the plant keepers were found to have significantly higher RAST scores for Fw, Fa, Fn, Fl and Fc but not for Fb, Fbs and Fer, although the difference was small. This might imply that the plant keepers had been exposed to some extent to the former plants. However, patient No.13, for example, had a convincing history of long-standing exposure only to Fb. In spite of this, IgE antibodies to all the other *Ficus* plants, except Fer, were detected in his serum. This speaks in favour of cross-reactivity between Fb and the other plants.

As the other *Ficus* plants contain Fb allergen, they might elicit allergic symptoms in Fb-sensitized subjects and also be potential sensitizers.

In RAST inhibition studies it is easy to interpret the results if there is only one allergen-antibody specificity involved. However, if there are several allergens in the extract, the situation is more complex and the inhibition curve tends to smooth out and/or to decline stepwise. The allergenic composition of the *Ficus* genus is difficult to evaluate, since several allergens are present. In a previous study (4) of weeping fig sap, 11 allergenic components were identified by SDS-PAGE and the immunoblotting technique.

Fb extract completely inhibited the RAST to the other *Ficus* discs in three of the investigated sera. An exception was patient No.2, whose serum had a somewhat different RAST profile than the other investigated sera (Table 1). This could either be due to a different *Ficus* exposure or an individual IgE-response to Fb allergens. His RAST reactions to Fw, Fl and Fc could be inhibited by only 50-74% by Fb extract. This patient's serum was previously investigated in a

study on characterization of allergens in weeping fig sap (4) and it was the only serum that recognized a distinct band at 20 kD. This might well explain the inhibition data and the slightly different RAST profile in this patient.

On the basis of the RAST inhibition capacity the eight *Ficus* extracts could be divided into three groups, namely: Fb and Fbs with a very good inhibitory capacity, Fw, Fn, Fl and Fc with an intermediate capacity and Fer with a low capacity. This indicates that Fbs also contains all relevant Fb allergens. The intermediate group is less potent and probably lacks some Fb allergen(s) which for Fer is more pronounced. It is not possible to evaluate the presence of unique allergens in any other *Ficus* than Fb in this study, since we do not have access to any person primarily sensitized to any of the other *Ficus* species. However, our clinical epidemiological investigations (2, 3) indicate that Fb, at least in Sweden, is predominant as an allergen source among the *Ficus* species used as indoor plants. If some *Ficus* plant is to be recommended for interior decoration, *Ficus elastica* "robusta" is probably preferable, due to the properties of the leaves; they have small total area, are easy to clean from dust and have thick cuticula.

In conclusion we found in this study that all other seven investigated *Ficus* plants contain various amounts of allergens common to Fb.

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