

Sensitization to *Ficus benjamina*: relationship to natural rubber latex allergy and identification of foods implicated in the Ficus-fruit syndrome

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Summary

Background Ornamental *Ficus benjamina* (FB) has been recognized as a new indoor allergen. Little is known about the prevalence in moderately exposed subjects and the proposed association with fruit and *Hevea* latex hypersensitivity.

Objective To study the prevalence of FB sensitization and the relationship with *Hevea* latex allergy, to identify cross-reacting fruits, and to characterize the responsible allergens.

Methods A skin prick test solution prepared from FB latex (200 µg/mL) was included in our routine screening programme for suspect inhalant allergy. Patients reacting with the FB extract were further skin tested with exotic fruits by the prick-to-prick method. Inhibition of fig and FB CAP by FB latex, fig (*Ficus carica*), kiwi, the thiolproteases ficin and papain, *Hevea* latex and rHev b 6.02 (hevein) was performed in selected patients.

Results Of 2662 patients with a positive skin test to any aeroallergen, 66 (2.5%) reacted with FB. Ten patients showed isolated sensitization to FB. Although FB-positive subjects were more often co-sensitized to *Hevea* latex than FB-negative (10.6% vs 3.8%, $P < 0.01$), nearly 90% tested negative for *Hevea* latex. Sensitization to FB was specifically associated with positive skin tests to fresh fig (83%), dried fig (37%), kiwi fruit (28%), papaya (22%), avocado (19%), banana (15%), and pineapple (10%) ($n = 54$). Clinical reactions were reported mainly from fresh and dried fig and kiwi (47%, 60%, and 64%, respectively, of skin test-positive patients), including seven patients with systemic reactions (urticaria, angioedema, asthma). CAP to fig in 11 patients with clinical fruit allergy was inhibited on average by 87% by FB latex, 89% by fresh fig, 80% by dried fig, 38% by kiwi (100 µg/mL each), and by 59% and 44% by ficin and papain (50 µg/mL), respectively. No inhibition was obtained with *Hevea* latex and rHev b 6.02. CAP to FB was inhibited on average by 95% by FB, 60% by fresh fig, 41% by ficin, 29% by papain, and less than 7% by rubber latex allergens.

Conclusions Sensitization to FB latex is found in 2.5% of atopic individuals and mostly occurs independently of *Hevea* latex allergy. Sensitization is commonly associated with allergic reactions to figs and other tropical fruits ('Ficus-fruit syndrome'). This cross-reactivity is mediated at least in part by thiolproteases.

Keywords *Ficus benjamina*, *Ficus carica*, fig fruit, ficus-fruit syndrome, hevein, kiwi fruit, latex-fruit syndrome, ornamental plants, papain, thiolproteases

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Introduction

Ornamental *Ficus benjamina* (FB) has been first recognized as a cause of respiratory allergy in occupationally exposed greenhouse workers [1]. More recent data indicate that also moderate exposure to this plant in private homes or offices may cause sensitization [2–4]. Rhino-conjunctivitis, breathing difficulties, and even severe anaphylaxis after contact with *Ficus* plants has been reported [1, 4, 5, 7]. Because FB allergens, like dust mite and cat allergens, accumulate in the house dust over time [3, 8], symptoms may take a relapsing or

chronic course, with patients not always being aware of the plants as the cause of their problems [9]. The prevalence of sensitization to FB has been insufficiently investigated until now.

In addition to the role of FB as a new, and possibly underestimated, indoor allergen, evidence has accumulated that sensitization to this plant is associated with adverse reactions to certain fruits, particularly fig fruit (*Ficus carica*), which belongs to the same plant genus [7, 10–13]. *In vitro* cross-reactivity between FB latex and fig fruit extracts has been demonstrated [7, 11–14]. The available data suggest that these patients were first sensitized by inhalation to airborne FB latex allergens and subsequently reacted to figs due to the presence of cross-reactive allergens in the fruit's pulp and skin, a condition that may be referred to as the 'Ficus-fruit

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syndrome'. Apart from fig fruit, the Ficus-fruit syndrome (FFS) may also comprise kiwi fruit, banana, and papaya [7, 11]. However, this assumption, although supported by clinical and *in vitro* data, is based on a limited number of patients only.

Another important, but still ambivalent, point in FB allergy is the proposed relationship with rubber latex (*Hevea brasiliensis*). *In vitro* data indicate a highly significant association between FB and rubber latex [15, 16]. Up to 60% of patients with rubber latex allergy have been reported to react to FB in the skin test [14–16] suggesting that sensitization to FB commonly results from cross-sensitization. Consistent with this view, a 45 kDa protein with 76% N-terminal sequence identity with rubber latex hevein (Hev b 6.02) has been identified recently as a cross-reactive allergen in FB latex [16]. As hevein-like domains of fruit class 1 chitinases constitute major allergens in the latex-fruit syndrome [17], it appears possible that *Ficus*-associated fruit hypersensitivity is not a distinct entity but is part of the latex-fruit syndrome. However, when looking at patients with documented FB or fig hypersensitivity it becomes evident that concomitant sensitization to rubber latex is not at all common [7, 10, 11, 18]. This questions the clinical relevance of cross-reactive hevein-like structures in FB allergy and *Ficus*-associated fruit hypersensitivity. Instead, thiolproteases have been suggested as relevant allergens [7, 11].

The aim of the present study was to evaluate the prevalence of sensitization to FB latex allergens in patients with suspect inhalant allergy by including a FB skin prick test (SPT) solution into our screening tray and to analyse the relationship with sensitization to rubber latex and common inhalant allergens. The foods involved in the FFS were identified by prick-to-prick testing with selected fruits and nuts in patients with a positive skin test to FB. To get more insight into the potential role of thiolproteases, CAP inhibition experiments were performed using purified ficin and papain as inhibitors (CAP-RAST, Pharmacia Diagnostics, Uppsala, Sweden).

Material and methods

Allergen extracts

A FB allergen extract was prepared by cutting leaves and twigs from one FB tree and picking up the extruding sap (latex) by means of a small pipette. The freshly collected latex was dissolved immediately in physiological saline. After centrifugation at 24 000g, the supernatants were dialysed with a cut-off of 6000–8000 Da and the total protein content determined following Bradford [19]. To prevent protein degradation, a standard cocktail of enzyme inhibitors including antificin was added (2 mmol ethylenediaminetetraacetic acid, 10 mmol disodium diethyldithiocarbamate, 1 mmol phenylmethyl sulphonylfluoride, 1 mmol leupeptin, 1 mmol antificin). The lyophilized extracts were re-suspended in distilled water, sterile filtered through a 0.22 µm MillexGV filter (Millipore, France), and stored as a stock solution divided into several vials at –20 °C. Prick test solutions were prepared over the whole study period from the same stock by dilution with a mixture of phosphate-buffered saline and glycerol (1:1, v/v) to achieve a final protein concentration of

200 µg/mL. Test solutions were preserved by 0.4% phenol. Extracts from fresh and dried figs and from kiwi fruit used for CAP inhibition experiments were prepared after the same extraction protocol.

The *Hevea* latex extract was a mixture of B- and C-serum proteins extracted from freshly harvested latex by ultracentrifugation at 40 000g at 4 °C for 1 h [20]. Hev b 6.02 was expressed in *Escherichia coli* as a recombinant protein fused to maltose binding protein as described in detail in reference [20].

Routine skin prick testing with *Ficus benjamina* extracts

The FB SPT solution was included into our routine allergy screening programme and tested together with pollens (birch, grass, mugwort, ragweed), house dust mite (HDM), moulds (*Cladosporium*, *Alternaria*, *Penicillium*), animal danders (cat, dog, horse, guinea-pig), and *Hevea* latex (all Soluprick, ALK, Hørsholm, Denmark) in all patients referred for suspect inhalant allergy.

Skin test reactions were judged as 2++ positive if the weal diameter was ≥3 mm, and as 3+++ if equivalent to or greater than the positive control (histamine dihydrochloride 10 mg/mL). Saline served as negative control. Skin reaction between 2 and 3 mm with surrounding erythema were classified as questionable positive (1+). Only 2++ and 3+++ positive reactions were counted as positive.

Prick-to-prick testing

Patients reacting with the FB extract in the routine test were invited to undergo a second skin test with selected foods to elucidate possible cross-sensitizations. These tests were made either immediately after the routine test or on a different day. The fruits tested by the prick-to-prick method were fresh and dried fig, mulberry (like *Ficus* also a member of the plant family Moraceae), kiwi, banana, pineapple, mango, papaya, avocado, grapes, kaki, potato, tomato, egg plant, and bell pepper. Peanut, almond, and soybean were tested by commercial extracts (ALK). Some of the foods were not tested in all patients. Simultaneously, patients' histories were taken regarding respiratory symptoms from FB exposure and adverse reactions to any of the tested foods.

CAP inhibition

CAP inhibition using fig fruit as well as FB as solid phase was carried out in 11 patients selected for a previous adverse reaction to either figs or other *Ficus*-associated fruits (patients 2–5 and 9–15 in Table 3). All had a positive skin test and a positive CAP to fig fruit and FB. Extracts from FB latex, fresh and dried fig, kiwi fruit, purified ficin from fig tree latex (Sigma-Aldrich, St Louis, MO, USA, F6008, lot no. 031K7665) and papain from papaya latex (Sigma-Aldrich, P4762, lot no. 031K7675), as well as *Hevea* latex and rHev b 6.02 (latex hevein), were used as inhibitors. Patients' sera were co-incubated with the respective inhibitor at 4 °C overnight before CAP (CAP-FEIA, Pharmacia, Sweden) was performed according to the manufacturer's recommendations. The final inhibitor protein concentration was 100 µg/mL for FB, fruit and rubber latex extracts, and 50 µg/mL for the single allergens. CAP inhibition using different inhibitor

concentrations (1, 10, 100, and 1000 µg/mL) was done with a serum pool prepared from the sera of patients 3, 10, 12, and 14 (selected for high IgE concentrations to FB and positive CAP to papain).

Results

Prevalence of *Ficus benjamina* sensitization in routine allergy screening

The FB extract was tested over a period of 16 months in more than 10 000 consecutive patients with suspect inhalant allergy. A databank was compiled from 4930 randomly selected patients and the prevalence of FB sensitization and the specific association with other allergens calculated from this sample.

Of the 4930 patients, 2662 (54%) reacted to any inhalant allergen in the routine test. Sixty-six of the 2662 patients (2.5%) showed a positive SPT to the FB extract. Isolated reactions to FB were encountered in ten patients (15.2% of positive FB tests), another two patients were positive to FB and rubber latex. The statistical association of FB with rubber latex and other allergens is summarized in Table 1. Sensitization to rubber latex was significantly more frequent in FB-positive patients than in FB-negative ($P = 0.0053$, χ^2 -test), but nearly 90% of them did not react with rubber latex. Of the 106 patients with a positive skin test to rubber latex, seven (6.6%) were positive also to FB. Combining rubber latex and FB-positive patients, only 4.2% (seven/165) were sensitized to both allergens.

No clear-cut correlations were observed with other inhalant allergens except molds ($P < 0.01$, χ^2 -test). Patients with a positive skin test to FB showed significantly more often multiple sensitization to indoor allergens ($P < 0.001$, χ^2 -test).

Table 1. Concomitant sensitization to *Hevea* rubber latex and common inhalant allergens as revealed by skin prick testing in 2662 patients with ($n = 66$) or without ($n = 2596$) sensitization to *Ficus benjamina* (FB) latex allergens

Concomitant sensitization to	FB negative $n = 2596$ (97.5%)		FB positive $n = 66$ (2.5%)		χ^2
	n	%	n	%	
Birch pollen	1057	40.7	32	48.5	NS
Grass pollen	1502	57.9	34	51.5	NS
Mugwort pollen	542	20.9	14	21.2	NS
House dust mite	983	37.9	26	39.4	NS
Animal dander	1181	45.5	34	51.5	NS
Moulds	171	6.6	11	16.7	0.0014
Rubber latex	99	3.8	7	10.6	0.0053
≥ 3 pollens	358	13.8	11	16.7	NS
Any indoor allergen	1710	65.9	45	68.2	NS
≥ 3 indoor allergens	306	11.8	17	25.8	0.0006

NS, not significant.

Cross-sensitization to fruits as revealed by prick-to-prick testing

Cross-sensitization to fruits was evaluated by prick-to-prick test in 54 patients with a positive SPT result to FB, including four patients with fig fruit allergy already described in reference [7] (patients 3, 4, 11, and 12 in Tables 3 and 4). Thirty-seven of the 54 patients were also positive to FB in the CAP. In all, 48 of the 54 patients reacted to any of the foods tested, with fresh fig provoking positive reactions in 45 (83%). The remaining foods were positive in 4–37% of patients, but no particular allergen was evidently associated with FB. Only five patients (9%), one of whom had clinical latex allergy (patient 13), reacted to rubber latex (Table 2).

Because 24 patients had concomitant sensitization to pollens, some positive skin reactions may have been due to cross-reactivity with pollens but not FB. When analysing the data separately for the 30 patients without pollen allergy, only fresh and dried fig, papaya, kiwi, banana, pineapple, and avocado gave positive results (Table 2). These fruits were considered to be specifically associated with FB. Rubber latex was positive in only one out of these 30 patients (3%). Considering all 54 patients, fresh fig was most often positive (83%), followed by dried fig (37%), kiwi (28%), papaya (22%), avocado (19%), banana (15%), and pineapple (10%).

Table 2. Positive skin test reactions to foods, pollens, and rubber latex in 54 patients with sensitization to *Ficus benjamina* (FB)

	Positive skin test: all patients ($n = 54$)		Positive skin test: patients without sensitization to pollens ($n = 30$)		Symptoms on exposure	
	n	%	n	%	n	%
Fresh fig	45/54	83	23/30	77	8/17	47
Dried fig	20/54	37	9/30	30	6/10	60
Mulberry	14/42	33	0/26	0		
Kiwi	15/54	28	7/30	23	9/14	64
Peanut	13/46	28	0/26	0		
Almond	11/47	23	0/26	0		
Papaya	12/54	22	7/30	23	1/2	50
Grapes	8/37	22	0/25	0		
Avocado	10/54	19	3/30	10	0/7	0
Potato	9/50	18	0/30	0		
Banana	8/54	15	4/30	13	3/8	38
Tomato	7/51	14	0/30	0		
Pineapple	5/52	10	2/30	7	1/5	20
Mango	5/51	10	0/30	0		
Soybean	3/43	7	0/25	0		
Kaki	2/40	5	0/27	0		
Egg plant	2/49	4	0/30	0		
Birch	17/54	31				
Grass	18/54	33				
Mugwort	11/54	20				
<i>Hevea</i> latex	5/54	9	1/30	3	1/4	25

Twenty-four of the 54 patients had concomitant sensitization to pollens. Skin responses were evaluated separately for the 30 patients without pollen sensitization to identify the fruits specifically associated with FB. Data on symptoms refer to anamnestic data in patients with a positive skin test to the respective allergen and previous exposure to this allergen.

Exposure to Ficus benjamina and clinical relevance of sensitization

Among the 54 patients studied by prick-to-prick test, 48 confirmed current or previous exposure to FB trees. Of the six remaining subjects, one primarily suffered from kiwi allergy and another one regularly used papain-containing meat tenderizers. A third patient, most strongly reacting with papaya in the skin test, suffered from intervertebral disc herniation once treated by surgery. The patient did not know whether chemonucleolysis using papain/chymopapain had been performed ever before.

Nineteen patients (35%) were aware of immediate symptoms after close contact with FB trees, in another 13 patients (24%) FB was a potentially relevant allergen. Thirteen patients (24%) denied problems from FB, and nine (17%) had no current contact with *Ficus* plants. Ocular and nasal symptoms were most common (68% and 53%, respectively). Contact urticaria, breathing difficulties, and angioedema occurred in four, two and one patient, respectively.

Clinical relevance of positive skin tests to Ficus benjamina-associated fruits

The potential clinical relevance of positive skin test results to FB-associated foods was evaluated by questionnaire in all 54 patients undergoing prick-to-prick testing. Altogether, 17 patients reported adverse reactions suggestive of type 1 allergy to at least one of the *Ficus*-associated fruits to which they had a positive skin test. The positive predictive value of a positive skin test result in those patients having eaten the respective fruit within the last year was 47% for fresh fig, 60% for dried fig, 64% for kiwi, 50% for papaya, 38% for banana, and 20% for pineapple (Table 2). No adverse reactions from avocado were reported. There may be under-reporting of allergic reactions to fresh figs and papaya because these fruits were only rarely eaten by our patients. All 17 patients reported mild-to-severe oral allergy syndrome, in seven patients also systemic symptoms occurred (Table 3).

CAP inhibition

Results from CAP inhibition experiments in 11 patients with clinical fruit allergy are presented in Table 4 and in Fig. 1. CAP to fig fruit was inhibited equally good by fresh fig and FB latex (fig: mean 89.3/median 94.7%; FB: 86.9%/92.5%), followed by dried fig (79.9%/78.8%) and ficin (58.6%/58.5%). Inhibition by papain and kiwi fruit was weaker (44.2%/45.9% and 38.1%/45.7%, respectively) and varied considerably between patients. The serum from patient 2 was the only serum where IgE-binding to fig allergens was poorly inhibited by FB latex (32.9%). This patient showed quite strong inhibition by papain (69.5%), and had a much higher CAP score to papain and fig than to FB. We assume that this patient was originally sensitized to papain. *Hevea* latex and rHev b 6.02 did not inhibit significantly IgE-binding to fig fruit in any of the patients (Table 4, Fig. 1a).

Inhibition of FB CAP (Table 4, Fig. 1b) showed a very similar pattern but complete inhibition was achieved only with FB itself (mean 94.8%/median 95.5%). In agreement with our previous observations [7], inhibition of FB CAP by fig fruit and ficin was only partial (fig: 60%/64%, ficin:

40.5%/41.7%). Inhibition by papain was mostly moderate (28.5%/23.4%) except in patient 2 where antibody binding was completely inhibited. None of the sera except that from patient 13 showed significant inhibition of the FB CAP by *Hevea* latex or rHev b 6.02.

Dose-dependent inhibition of IgE-binding to FB and fig fruit of a serum pool prepared from the sera of four patients with a positive CAP to fig fruit and papain is shown in Fig. 2. CAP to FB was inhibited by more than 80% by 1 µg/mL of FB extracts whereas comparable inhibition could be achieved neither with fig fruit nor the other inhibitors at concentrations as high as 1000 µg/mL. In the fig fruit CAP, the inhibition curve for FB latex was similar to that of fig fruit. Maximum inhibition by ficin and papain was 83.7% and 53.4%, respectively. In agreement with the inhibition data for individual sera, *Hevea* latex never inhibited significantly antibody binding of the serum pool to either FB or fig fruit.

Discussion

Sensitization to certain inhalant allergens may be associated with secondary food hypersensitivity due to the occurrence of cross-reactive allergens in some foods. Pollens are the most common elicitors of secondary food allergies [21], but HDM and rubber latex allergy may be associated as well with particular food hypersensitivities [22, 23]. Here we provide evidence for the existence of a new syndrome, the FFS, which is assumed to have its origin in primary respiratory sensitization to FB latex allergens and succeeding cross-sensitization to various fruits. We identified in this study fig, kiwi, banana, papaya, pineapple, and avocado as important foods implicated in this syndrome, and could demonstrate cross-reactivity between FB latex and some of these fruits *in vitro*.

Almost 90% of our patients were keeping or had kept previously a FB tree in their homes, advocating for the importance of exposure to *Ficus* plants in the private environment as the origin of sensitization. There may be, however, some cases where fruit or papain allergy may have preceded FB allergy, e.g. in patient 2. The proposed role of FB as the primary sensitizer in the FFS is also supported by the observation that antibody binding to fig fruit can be inhibited completely by FB latex in most patients, whereas reactivity with FB latex is only partially inhibited by fig proteins even at very high concentrations [7, 12].

Fig clearly is the fruit most closely associated with FB allergy and it also accounts for most of the severe adverse reactions observed in our patients. Sensitization appears to be clinically relevant in approximately half of sensitized subjects, if exposed, but presumably remains latent in the many patients never eating figs. Dried fig elicited fewer positive skin test reactions than fresh fig, suggesting that some allergenic components are lost during the drying process. However, in the light of patients' histories and results from CAP inhibition, it seems that it is just the clinically important allergens that remain preserved. In consistency with this view, 16 of the 17 patients reporting fruit allergy had at least a weakly positive skin test to dried fig. Therefore, a positive skin response to dried fig may signify a high risk for developing allergic reactions to one of the *Ficus*-associated foods. Symptoms from the other fruits were less frequent and

Table 3. Skin test results and clinical symptoms in 17 patients with a history of adverse reactions to *Ficus*-associated foods

Patient	Skin test				Symptoms from				FB-associated fruits	Rubber latex	Other foods	Pollen sensitization	
	FB latex	Fresh fig	Dried fig	Kiwi	Papaya	Banana	Pineapple	Hevea latex					FB exposure
1	+++	++	+	-	-	-	-	-	Rhino-conjunctivitis, ocularoedema, sneezing	Angioedema, laryngeal oedema, asthma, nasal congestion from fresh figs	No	No	No
2	++	+++	+++	+++	+++	++	+	+	Sneezing, rhinorrhoea, ocular itch	Severe OAS, nausea, diarrhoea from fresh figs, severe OAS from kiwi and papaya	No	Kaki	No
3	+++	+++	+++	++	+	-	+	-	Nasal congestion, ocularoedema	Angioedema, nausea, vomiting, OAS from fresh figs, OAS from kiwi	No	Peach?	No
4	++	++	+	-	-	+	-	-	Sneezing	OAS and nasal congestion from fresh figs, mild OAS from kiwi and banana (?)	No	Chestnuts?	Mugwort
5	+++	+++	++	-	-	-	-	-	Contact urticaria, ocular itch	OAS from fresh figs	No	No	Birch, grass
6	++	++	++	+++	-	-	+	+	Rhino-conjunctivitis	OAS from fresh fig and kiwi	Itch (gloves, condoms)	Apple, cherry, carrot, nuts	Many
7	++	+	++	+	-	-	-	++	No	OAS and sneezing from fresh figs, kiwi, and pineapple	No	Apple, peach, carrot, nuts	Birch
8	+++	+++	+	-	++	++	-	-	Contact urticaria, ocular itch, sneezing, angioedema, asthma	OAS from fresh and dried figs and banana	No	No	Mugwort
9	+++	+++	++	-	+	++	-	-	No	Angioedema, severe OAS from dried figs	No	No	No
10	+++	+++	+++	++	+++	-	++	-	No	Urticaria, nausea, vomiting, asthma from dried figs, OAS from kiwi	No	Apple, carrot, nuts	Birch, grass, mugwort
11	++	++	++	+	+	+	+	-	Unknown	Angioedema, urticaria, nasal congestion from dried figs, OAS from pineapple?	No	No	Birch, mugwort
12	+++	+++	+++	++	++	-	+	-	Conjunctivitis, ocularoedema	Severe laryngeal oedema and breathing difficulties from dried figs	No	No	Birch
13	++	+++	+++	+++	+++	+++	++	++	Sneezing, rhinorrhoea	Angioedema, urticaria, laryngeal oedema, asthma, conjunctivitis from kiwi and banana, OAS and rhinitis from pineapple	Contact urticaria (gloves)	Peach, flour?	No
14	+++	+++	+++	++	+++	+	-	-	Contact urticaria, rhino-conjunctivitis	OAS from kiwi and pineapple	No	No	No
15	+++	+++	++	++	++	++	++	-	Conjunctivitis, ocularoedema	OAS from kiwi and banana	No	Belipepper?	No
16	++	+++	+++	++	-	-	-	-	Sneezing, rhinorrhea	OAS with mild laryngeal oedema from kiwi and pineapple	No	Apple, peach, grapes, cherry, carrot, nuts	Birch, grass, plantain
17	++	++	-	+++	-	-	-	-	No exposure	OAS from kiwi	No exposure	No	No

FB, *Ficus benjamina*; OAS, oral allergy syndrome; skin test results: +++ weal diameter ≥ 3 mm, ++ weal diameter ≥ 3 mm, + questionable positive, weal diameter 2–3 mm.

Table 4. Inhibition of CAP to fig fruit and *Ficus benjamina* (FB) in 11 patients with a positive skin test to fig fruit and a history of an allergic reaction to figs or other *Ficus*-associated fruits (for details see Table 3)

Patient	CAP (kU/L)										Inhibition of fig fruit CAP (%)										Inhibition of FB CAP (%)									
	CAP (kU/L)					Hevea latex					Hevea latex					Hevea latex					Hevea latex					Hevea latex				
	FB	Fig	Papain	Papain	Fig	FB latex	Fig latex	Papain	Hevea latex	Hevea latex	FB latex	Fig latex	Papain	Hevea latex	Hevea latex	FB latex	Fig latex	Papain	Hevea latex	Hevea latex	FB latex	Fig latex	Papain	Hevea latex	Hevea latex	FB latex	Fig latex	Papain	Hevea latex	Hevea latex
2	2.2	11.3	16.9	<0.35	<0.35	32.9	90.5	77.8	20.0	46.9	69.5	0	0	0	93.1	79.7	49.0	93.1	7.9	3.4	93.1	49.0	93.1	7.9	3.4	93.1	49.0	93.1	7.9	3.4
3	22.3	13.7	24.9	<0.35	<0.35	97.6	99.2	92.8	32.8	62.1	45.9	0	0	0	95.1	64.9	41.7	24.7	11.4	6.8	95.1	41.7	24.7	11.4	6.8	95.1	41.7	24.7	11.4	6.8
4	2.4	1.8	<0.35	<0.35	<0.35	95.8	95.8	62.7	6.6	55.3	1.6	6.7	0	0	96.7	49.4	21.3	12.3	0.8	4.6	96.7	21.3	12.3	0.8	4.6	96.7	21.3	12.3	0.8	4.6
5	60.0	9.6	<0.35	<0.35	<0.35	91.0	69.0	55.2	26.6	27.8	18.5	1.8	0	0	96.2	80.3	49.7	0	10.6	0	96.2	49.7	0	10.6	0	96.2	49.7	0	10.6	0
9	8.4	2.3	3.3	<0.35	<0.35	83.1	68.0	65.7	47.8	55.6	61.1	0.7	0	0	93.5	39.8	10.9	20.2	9.8	0	93.5	10.9	20.2	9.8	0	93.5	10.9	20.2	9.8	0
10	100.0	29.3	22.6	<0.35	<0.35	89.7	88.6	77.1	47.6	49.5	34.5	0	0	0	95.5	74.5	62.7	30.3	0	12.5	95.5	62.7	30.3	0	12.5	95.5	62.7	30.3	0	12.5
11	90.4	3.6	<0.35	<0.35	<0.35	96.2	96.2	89.1	0	58.5	37.1	0	0	0	97.0	50.2	33.8	ND	ND	ND	97.0	33.8	ND	ND	ND	97.0	33.8	ND	ND	ND
12	60.8	5.0	3.4	<0.35	<0.35	94.7	94.6	89.1	55.8	59.0	52.5	0	0	0	95.9	29.9	16.7	2.7	0	4.1	95.9	16.7	2.7	0	4.1	95.9	16.7	2.7	0	4.1
13	69.5	21.7	38.2	2.5	85.8	85.0	79.8	79.8	45.7	59.3	55.9	0.3	11.4	0	89.7	75.8	61.2	62.9	23.2	10.1	89.7	61.2	62.9	23.2	10.1	89.7	61.2	62.9	23.2	10.1
14	97.7	41.5	20.9	<0.35	<0.35	92.5	98.7	92.8	52.0	94.2	43.4	0	0	0	93.5	51.4	39.9	24.0	5.3	4.0	93.5	39.9	24.0	5.3	4.0	93.5	39.9	24.0	5.3	4.0
15	16.8	3.2	2.8	<0.35	<0.35	96.4	96.6	97.3	84.2	76.5	65.7	2.3	0	0	98.1	64.0	59.0	22.9	9.8	0	98.1	59.0	22.9	9.8	0	98.1	59.0	22.9	9.8	0
Mean $\pm$ SD						86.9 $\pm$ 18.5	89.3 $\pm$ 11.2	79.9 $\pm$ 13.9	38.1 $\pm$ 24.1	58.6 $\pm$ 16.7	44.2 $\pm$ 20.5	1.1 $\pm$ 2.0	1.0 $\pm$ 3.4	0 $\pm$ 2.6	60.0 $\pm$ 17.0	40.5 $\pm$ 18.1	28.5 $\pm$ 29.4	6.7 $\pm$ 8.8	2.7 $\pm$ 7.1		60.0 $\pm$ 17.0	40.5 $\pm$ 18.1	28.5 $\pm$ 29.4	6.7 $\pm$ 8.8	2.7 $\pm$ 7.1	60.0 $\pm$ 17.0	40.5 $\pm$ 18.1	28.5 $\pm$ 29.4	6.7 $\pm$ 8.8	2.7 $\pm$ 7.1

Patients' sera were pre-incubated at 4 °C overnight with allergenic extracts from FB latex, fresh and dried fig, kiwi fruit, the thiolproteases ficin (from fig tree latex) and papain (from papaya latex) as well as with natural rubber latex extract and rHev b 6.02 (latex hevein). The final inhibitor concentration was 100 µg/mL for crude extracts, and 50 µg/mL for single proteins. Figures represent percent inhibition of CAP IgE binding in comparison with the non-inhibited control.

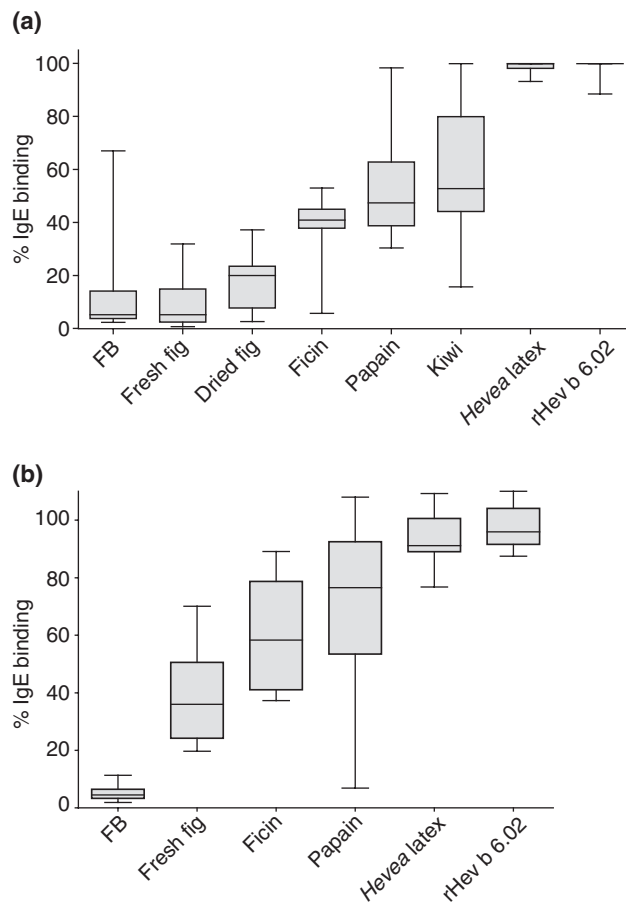


Fig. 1. Inhibition of CAP IgE-binding to fig fruit (a) and *Ficus benjamina* (b) in 11 patients with allergic reactions to figs or other *Ficus*-associated fruits (same patients as in Table 4). Box plots show median percent inhibition and interquartile range. Sera were pre-incubated with extracts from *Ficus benjamina* (FB) latex, fresh and dried fig, kiwi fruit, the thiolproteases ficin and papain, as well as with natural rubber latex extract and rHev b 6.02 (latex hevein). Final inhibitor concentrations were 100 µg/mL for crude extracts, and 50 µg/mL for single proteins.

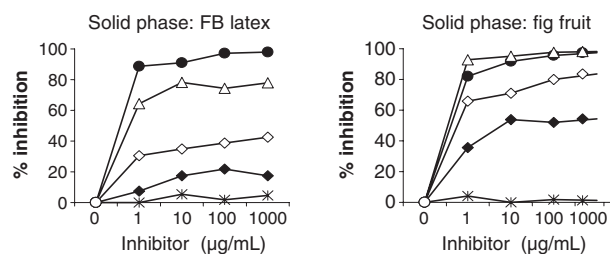


Fig. 2. Dose-dependent inhibition of CAP IgE-binding to *Ficus benjamina* (FB) latex and fig fruit allergens of a serum pool prepared from four patients with IgE to fig fruit and papain (patients 3, 10, 12, and 14). Baseline CAP values of the non-inhibited serum pool were 13.95 kU/L for FB (serum dilution 1 : 3), and 16.9 kU/L for fig fruit (undiluted serum). •, FB latex; △, fresh fig fruit; ◇, ficin; ◆, papain; *, Hevea latex.

mainly confined to the oral cavity, but at least banana and kiwi may elicit systemic reactions, too. We guess that papaya also can cause severe systemic reactions as some patients showed very strong skin test reactions to this fruit and had high CAP scores to papain. However, due to the rare consumption of papaya in our clientele its significance must remain open at the moment.

Sensitization to mulberry (*Morus* spp.), which belongs to the same plant family as *Ficus* (i.e. Moraceae), is not part of the FFS but rather related to concomitant birch pollen allergy, corroborating our previous observations [7]. In contrast, another member of the Moraceae, *Artocarpus*, which delivers two important basic foodstuffs in tropical countries, breadfruit (*Artocarpus altilis*) and jackfruit (*A. heterophyllus*), could be involved in the FFS. DeGreef and colleagues described a road worker with banana hypersensitivity developing contact urticaria and rhinitis when cutting *Ficus* and *Artocarpus* trees [24]. Strong cross-reactivity between the plants' latexes was found *in vitro*. Nevertheless, breadfruit is less likely a relevant food allergen because it is not eaten raw, and its allergens may be assumed to be thermolabile as are the allergens in fig and FB latex [7, 18]. In accordance with this, the patient described by deGreef could eat cooked breadfruit without symptoms [24]. Jackfruit, however, which is also eaten raw as a ripe fruit, should be taken into account.

Despite FB having become a most popular ornamental pot plant all over the world and accumulating evidence for sensitization in moderately exposed subjects, little is known about the prevalence of sensitization. We skin tested in this study some thousand patients and found positive reactions to FB latex in 2.5%, a value comparable to that found for rubber latex. The higher frequency in FB-sensitized patients of multiple sensitization to other indoor allergens, and moulds in particular, may simply reflect marked atopy in these patients but might also indicate a higher risk to develop FB sensitization in case of other pre-existing indoor allergies. On the other hand, the finding of isolated sensitization in some patients underlines again that sensitization is not confined to atopics [4, 5].

Axelsson et al. [9] reported a prevalence similar to ours while other studies found much higher values between 7% and 18% [3, 14, 15, 25]. Apart from different exposure, a major reason for these discrepancies may be the material used for skin testing. Some investigators used crude allergen extracts with unknown protein content, possibly higher than in our extract, or pure FB latex which may provoke false-positive reactions [15]. We assume that our in-house preparation, adjusted arbitrarily to a concentration of 200 µg/mL, was highly specific as no irritant and few questionable positive reactions occurred. In patients with a clear-cut history of FB or fig allergy, the test solution provoked strong skin test reactions of 10 mm and higher, indicating also good sensitivity. We cannot exclude, however, that a higher protein concentration would have identified more patients, or that some low molecular weight allergens have been lost during dialysis. Unfortunately, prick test solutions from pure FB latex are not or not easily commercially available. To obtain comparable results from different populations and to disclose local differences in the prevalence of FB allergy commercial high-quality extracts are urgently needed.

It is evident that the fruits claimed here to constitute the FFS are largely identical with those involved in the latex-fruit syndrome [23]. In the latter, fruit class I chitinases displaying hevein-like domains have been identified as major cross-reactive allergens [17, 26, 27]. As fig fruit hypersensitivity has been described also in the context of latex-associated food

allergy, although not very often [28–30], it is well conceivable that hevein-like allergens in fig, similar to those identified in FB latex [16], are important allergens. However, there is sufficient evidence now from this and previous studies that latex allergy is not mandatory in *Ficus*-related allergies. We demonstrated in this study that the IgE reactivity to fig fruit allergens of patients with FB sensitization can be inhibited neither by *Hevea* latex nor by recombinant latex hevein, and in the FB CAP only one out of 11 sera showed some inhibition by rubber latex. Although the skin test results from 2662 atopics revealed a significant association between FB and rubber latex, it is much more striking that 90% of FB-sensitized subjects did not react with rubber latex. This contradicts other investigators finding cross-sensitization to FB in up to 60% of patients with rubber latex allergy [14–16]. A simple explanation for this disagreement would be that double sensitization to FB and rubber latex is common in a pre-selected population suffering from latex allergy where sensitization to Hev b 6 is frequent [16], but not in an unselected population with suspect inhalant allergy. Here FB allergy occurs mostly independent of rubber latex allergy, and probably other allergens are involved.

Thiolproteases have been suggested as major allergens in *Ficus*-related allergies because many patients with fig fruit allergy have IgE against papain, a thiolprotease from papaya [7, 11]. Immunologic cross-reactivity between different plant thiolproteases has been long known from animal studies [31]. Furthermore, thiolproteases from papaya (papain), pineapple (bromelain), and kiwi (actinidin), i.e. fruits claimed by us to be involved in the FFS, have been identified previously as important occupational inhalant allergens [32, 33] or food allergens [34]. Unfortunately, none of these studies addressed the potential association with FB. We now provide experimental evidence for the proposed role of thiolproteases as cross-reactive allergens in the FFS. A commercial ficin preparation from fig tree latex substantially inhibited IgE binding to fig allergens, even if this inhibition was always weaker than inhibition by crude fig extracts. It remains to be elucidated whether complete inhibition could be achieved with other ficin preparations (e.g. ficin from fig fruit) or if also allergens other than thiolproteases play a role. Anyhow, in the FB CAP inhibition, ficin and papain were only moderate inhibitors. This may well point to the involvement of additional allergens, at least in respiratory allergy to *Ficus* trees.

In conclusion, we found sensitization to FB allergens in 2.5% of atopics with inhalant allergy, and demonstrated specific cross-sensitization to fig fruit (*Ficus carica*), kiwi, papaya, banana or pineapple that may lead to local oral as well as systemic reactions ('FFS'). The FFS is a distinct entity largely independent of *Hevea* latex allergy. Thiolproteases such as ficin and papain, but not hevein-like proteins, appear to be important cross-reactive allergens in this syndrome.

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