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- Dr. R. Pérez-Calderón, Dr. M.A. Gonzalo-Garijo and Dr. FJ Rodríguez-Velasco designed the study.
- Dr. R. Pérez-Calderón, Dr. M.A. Gonzalo-Garijo and Dr. S. Sánchez-Vega contributed to selection and inclusion of the patients
- Prof. Bartolomé-Zavala performed laboratory tests
- All the authors: Analysis and interpretation of data, revision and approval of the text

ABSTRACT

BACKGROUND: Occupational respiratory diseases in workers of peach tree crops have been reported punctually and have been associated with sensitization to proteins present in both pollen and leaf tree. We report the study of 37 workers with respiratory symptoms related to occupational exposure to peach trees.

METHODS: Prick tests and specific IgE determinations were performed with extracts from leaves and branches of peach tree. Immunodetection in leaf extract was realized by SDS-PAGE-immunoblotting with patient sera and rabbit serum anti-Pru p 3. Immunodetection inhibition was performed with rPru p 3 and pollen profilins. The clinical relevance of sensitization was demonstrated by specific bronchial challenge test (SBCT) with peach leaf extract.

RESULTS: Most patients suffered symptoms when peach trees had leaves, specifically during thinning and harvesting fruit (rhinoconjunctivitis: 100% and asthma: 67.5%).

Sensitization to leaf extract was demonstrated in 86% of patients. IgE-immunoblotting with peach leaf extract revealed in 6 patient sera a pair of bands of 10 and 16 kDa, and in 9 a 16 kDa band. Those bands could be two isoforms of peach leaf LTP, so the recognition frequency of some LTP isoform by our patient sera was 42%. 33% of the sera recognized a doubled band of about 14.5 kDa and this recognition was inhibited by nPho d 2. The SBCT with peach leaf extract was positive in the asthmatic sensitized patients tested.

CONCLUSIONS: Sensitization to peach leaves was the cause of occupational respiratory symptoms in our patients. Some patient sera revealed IgE-binding proteins matching LTP and/or profilin.

KEY WORDS: lipid transfer proteins (LTP); occupational allergy; peach tree; profilin; respiratory allergy

INTRODUCTION

Allergens in the workplace can cause dermatitis, rhinoconjunctivitis and occupational asthma (1). Horticulturists and workers of fruit trees, vegetables, flowers and ornamental plants have higher risk of respiratory diseases. They are exposed to pollens, molds, mites, insects, environmental particles originated from cultivated plants, endotoxins and pesticides (2).

Rosaceae is a family of entomophilous dicotyledonous plant members of the order Rosales. They include subfamilies with considerable economic importance because most of the species of fruits commonly consumed in the world belong to them: apple, pear, quince,

peach, plum, cherry, strawberry, almond, apricot, blackberry, raspberry, etc. They also include many ornamental plants such as roses (3). *Rosaceae* fruits are one of the main causes of food allergy in the Mediterranean countries, associated or not to sensitization to pollens (4,5).

Occupational allergy due to *Rosaceae* pollen is rare. It may cause sensitization in exposed individuals and it has been described as a cause of occupational respiratory allergy in workers in strawberry crops (6) and roses (7), and in workers of artificial pollination of the Japanese pear (8) or peach tree (9).

Non-pollen structures of fruit trees, such as almond tree leaves and mesocarp and epicarp of almonds, act as a source of airborne allergens (10). Peach tree leaves were the cause of occupational respiratory allergy in a worker of a peach orchard (11) and in a worker of a peach-leaf analysis laboratory (12). Both cases were associated with sensitization to lipid transfer proteins (LTP) present in allergenic extracts made from leaves. *Rosaceae* fruit handling was the cause of occupational respiratory allergy in a factory worker (13) and exposure to large amounts of fruits (including peaches) in a wholesale fruit storehouse produced the same symptoms in an administrative employee (14). A case of anaphylaxis by inhaled sensitization to peach LTP (Pru p 3) has recently been reported in a patient living in an orchard (15).

It is known that an allergenic source may express different isoforms of the same allergen, but this has rarely been described for LTPs. Bolla *et al.* demonstrated that the use of Acid Urea-PAGE combined with SDS-PAGE can be considered a valid approach for the separation of LTP isoforms. They showed that pomegranate expresses different LTP isoforms characterized by different IgE reactivity (16). Martín-Pedraza *et al.* showed the existence of LTPs isoforms with different IgE-binding activity in peel and seed from tomato (17).

In Extremadura, there are currently about 40,000 hectares of fruit trees, with a density of about 800-1000 trees per hectare (18). The flowering period lasts between 10-25 days (19) and, at this stage, workers do not usually work in the trees. Pruning, thinning and harvesting fruit are done manually and require close contact of workers with leaf trees.

Based on these data and on the clinical observation that some of our patients reported respiratory symptoms with occupational exposure to peach trees (specifically during thinning and harvesting fruit), we proposed this study in order to assess whether the leaves and branches of peach trees were the sources of inhalant allergens in a sample of patients diagnosed with seasonal occupational rhinoconjunctivitis and asthma.

METHODS

Patients

The sample selection was carried out by an intentional non-probabilistic sampling among patients diagnosed in our consultations with rhinoconjunctivitis and/or seasonal asthma according to clinical practice guidelines (20,21). The only inclusion criterion was patients had symptoms only after exposure to peach trees or worsened their seasonal symptoms after the work performed through the peach trees for at least one season work. Exclusion criteria were age under 18 years, pregnancy, immunological or neoplastic diseases and psychiatric illnesses that prevent understanding the nature of the study. All patients selected to participate in the study signed an informed consent document approved by the Ethics Committee of Clinical Research of our Hospital.

Besides the usual anamnesis, all patients underwent a specific questionnaire that included: number of years of exposure to fruit trees, years since onset of symptoms, months

of the year when symptoms occurred, work performed in fruit trees and tasks related to the onset of symptoms.

Cutaneous tests

Skin prick tests were performed according to recommendations of Global Allergy and Asthma European Network (GALEN2) and Allergic Rhinitis and Its Impact on Asthma (ARIA) (22) with a battery of inhalant allergens including house dust and storage mites, pollens of grasses, trees and weeds, animal dander, molds (ALK-Abelló, Madrid, Spain) and *Tetranychus urticae* (Roxall España, Bilbao, Spain). In addition, we performed prick tests with date palm profilin (Pho d 2, 50 µg/ml) (ALK-Abelló, Madrid, Spain), peach LTP (Pru p 3, 30 µg/ml) (ALK-Abelló, Madrid, Spain) and with extracts of leaves and branches of peach tree prepared by us.

Extracts from leaves and branches of peach tree

For the preparation of the extracts, leaves and branches of peach trees were collected at the time of harvesting in our area. To avoid contamination with fungi or mites, leaf collection was conducted by an expert agricultural engineer, who selected leaves with no signs of pests or diseases on plantations untreated with pesticides in the past 6 weeks. The extracts were prepared in laboratories Roxall España (Bilbao, Spain) according to the method previously described (23). Prick extract concentration was 10 mg/ml.

Total and Specific IgE

Total IgE was determined by using the CAP-FEIA System (Pharmacia, Uppsala, Sweden) following the manufacturer's instructions. Specific IgE to extracts of branch and leaf peach, Pru p 3, and pollen profilin from *Artemisia vulgaris* (nArt v 4), *Phoenix dactylifera* (nPho d 2) and *Platanus acerifolia* (nPla a 8) were performed following recommendations of National Committee for Clinical Laboratory Standards (NCCLS) (24). The EAST (Enzyme AllergoSorbent Test) technique was used, and the results were expressed in accordance with the manufacturer's instructions (Hytec-specific IgE EIA; Hycor Biomedical Inc., Irvine, California, USA). The extracts (10 mg/ml [wt/vol]) were coupled to cyanogen-activated paper discs, as described by Ceska and Lundkvist (25).

Immunoblotting and Immunoblotting inhibition assays

Specific IgE immunodetection by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE immunoblotting) was performed according to the method of Laemmli (26) using 12.5% and 4% acrylamide as separating and stacking gels, respectively. The assay was performed in reducing (with 2-mercaptoethanol) and non-reducing conditions (without 2-mercaptoethanol). Twenty micrograms of protein was applied per lane. Separated protein bands were electrophoretically transferred to polyvinylene difluoride, essentially as described by Towbin et al. (27). Membranes were incubated overnight at 4°C with patient serum samples, followed by anti-human IgE–horseradish peroxidase conjugate incubation, and detected by a chemiluminescence method (ECL-Plus; Amersham Pharmacia Biotech, Uppsala) as recommended by the manufacturer.

Immunoblotting inhibition tests of IgE binding to the leaf peach extract were performed by preincubating patient serum samples with the inhibitor phases Pru p 3 (75 and

100 µg/ml), pollen profilins (100 µg/ml) from *Artemisia vulgaris* (nArt v 4), *Phleum pratensis* (rPhl p 12), *Phoenix dactylifera* (nPho d 2), *Olea europaea* (rOle e 2) and *Platanus acerifolia* (nPla a 8) overnight at 4°C before exposure to the membranes. A pool of serum samples from non-atopic subjects was used as a negative control for *in vitro* tests.

In order to show whether the peach leaf extract contained a protein homologous to rPru p 3, the extract was analysed by SDS-PAGE immunoblotting with rabbit serum anti-Pru p 3.

Specific bronchial challenge test

Specific bronchial challenge test (SBCT) was performed with the peach leaf extract following the procedure previously described (28). All tests were performed by a continuously pressurized nebulizer (DeVilbiss 646; DeVilbiss Co, Somerset, PA) with output of 0.28 ml/minute and a particle size range of 0.5 to 2 microns. To determine the initial concentration of challenge, we performed endpoint titration prick tests with the leaf extract prepared for bronchial challenge (the highest concentration was 5 mg/ml). SBCTs were performed when patients were asymptomatic and without occupational exposure.

Aerosolized particles generated by the nebulizer DeVilbiss 646 were inhaled for 2 minutes at normal breathing volume, starting with the control PBS solution and followed, at 30-minute intervals, by progressive concentrations of the peach leaf extract. Pulmonary function tests were performed 10 and 30 minutes after each dose. A positive response was defined as a FEV1 decrease greater than 20%. If the test was negative, the next dose was 5 times higher. After SBCT, hourly peak expiratory flow measurements were recorded for 9 hours. The results were expressed in extract concentration (mg/ml) causing a decrease in FEV1 of 20% compared to that obtained after the diluent.

RESULTS

Characteristics of the sample

We studied 37 patients with a mean age of 40.3 years (range 18-67). 64.9% of them were females and 34.1% males. The mean of exposure to peach trees was 10.5 ± 8.7 years (range 1-40), and the mean of symptom onset was 7.9 ± 5.7 years (range 1-20). 94.6% of patients worked on collecting fruit and 46.8% also worked on the thinning of fruit. In addition, some patients worked in dry pruning (n: 2), green pruning (n: 25) and handling of peaches in store for selection and calibration (n: 3). Patients had symptoms only, and/or worsening of their respiratory symptoms when working with trees with leaves. No patient had symptoms when the trees had no leaves or handling fruit, but all of them had symptoms during harvesting.

All patients had rhinoconjunctivitis (18.9% mild, 59.5% moderate and 21.6% severe) and in the majority of the cases it was persistent (n= 33). 67.5% of patients (n= 25), had asthma (mild in 5 patients, moderate in 8, and severe in 12), partly controlled in 14 of them.

Eight patients (22%) reported contact urticaria in relation to thinning and harvesting of peaches (P3, P6, P8, P16, P22, P23, P24, P29). Patients P3 and P6 presented cutaneous symptoms before respiratory symptoms and, in the rest, both symptoms began simultaneously. All patients tolerated the ingestion and manipulation of peaches.

Ten patients (37%) had food allergy, most by *Rosaceae* fruits. Oral allergy syndrome (OAS) was the most frequent manifestation (7 patients) due to peach (P1, P3, P19, P20, P23, P36 and P37), plum (P1, P3, P19 and P20), and nectarine (P19). P4 suffered a systemic reaction with nectarine and almond. Non-*Rosaceae* fruits were responsible for a systemic

reaction with kiwi (P4) and OAS in 3 patients: melon and watermelon (P10), melon (P19 and P20) and mango (P23).

Cutaneous tests and specific IgE (Table I)

Thirty-two (86%) of the 37 patients studied were sensitized to peach leaf extracts with positive skin test (21: 57%) and/or positive specific IgE > 0.35 kU/L (30: 81%). Skin prick test with the peach branch extract only proved positive in 6 patients. Specific IgE determination against peach branch extract was performed in 4 patients and was positive in all of them. All patients sensitized to branches were also sensitized to leaves. Prick test with *Tetranichus urticae* extract was positive in only 3 patients. Most of our patients were sensitized to some kind of pollen (23: 62%) and only 6 patients (16.2%) were monosensitized to peach leaves (Table 1). Prick test with Pru p 3 was positive in 10 patients (27%) and specific IgE to Pru p 3 was positive in 8 sera (21.6%). Prick test with Pho d 2 (pollen profilin) was positive in 10 patients (27%). Seven sera had specific IgE against profilin from *Artemisia vulgaris* pollen (nArt v 4) and 10 against profilin from *Platanus acerifolia* pollen (nPla a 8).

Specific bronchial challenge test (SBCT) results

SBCT with peach leaf extract was performed in 6 patients with rhinoconjunctivitis and asthma when exposed to peach trees. It was positive in 4 of them, all sensitized to leaf extract. In the 2 patients with negative results, skin tests with leaf extract were negative, although one of them had positive specific IgE against this extract. The results of these challenge tests are summarized in Table II. The response to SBCT was immediate in 2 patients, delayed in a patient and dual in another one. Delayed symptoms were assessed in a

medical emergency center and a decrease in peak expiratory flow (PEF) was observed in both cases.

SDS-PAGE immunoblotting results

SDS-PAGE immunoblotting with peach leaf extract was performed with 35 patient sera. When the assay was performed without 2-mercaptoethanol, 6 sera revealed a pair of bands of 10 and 16 kDa, and 9 sera only a 16 kDa band (Figure 1). But when the test was performed under reducing conditions with 2-mercaptoethanol, a single band about 10 kDa was observed in this zone of the gel with 7 patient sera (Figure 2). In assays with 2-mercaptoethanol, 34% of sera (n = 12) detected a double band of a somewhat larger size to 14.5 kDa (Figure 2). No IgE binding bands were detected with 11 patient sera that had specific IgE against leaf extract. Other bands about 28 to 66 kDa were identified with 15 patient sera under reducing conditions (Table 1).

Characterization of IgE binding bands

The 10 kDa and 16 kDa bands recognized in the peach-leaf extract by sera from some patients could correspond to two isoforms of LTP. To confirm this hypothesis, we carried out 2 studies: SDS-PAGE immunoblotting with anti-Pru p 3 rabbit serum, and SDS-PAGE immunoblotting inhibition with the sera of patients P4 and P18 and purified nPru p 3.

The anti-Pru p 3 rabbit serum detected two bands about 16 and 10 kDa when performing the test under nonreducing conditions (Figure 3). The molecular mass of these bands matches with the bands detected with the sera of 15 patients (Table 1). When performing the test under reducing conditions (with 2-mercaptoethanol), anti-Pru p 3 rabbit

serum detected a band of approximately 10 kDa (Figure 3). The molecular mass of this band coincides with one of the bands detected with the sera of 7 patients (Table 1).

The results of immunoblotting-inhibition without 2-mercaptoethanol when sera P18 and P4 were pre-incubated with nPru p 3 (100 ug/ml) showed a total IgE-binding inhibition to the 16 kDa and 10 kDa-bands (Figure 4). In the test performed with serum P18, inhibition also occurred with 75 ug/ml of nPru p 3. As positive control of inhibition, preincubation of the serum with peach leaf extract was used, and as negative control, preincubation with ovalbumin.

The results of both tests suggest that the bands about 16 and 10 kDa could be two isoforms of peach leaf LTP. Therefore, the recognition frequency of some LTP isoform by our patient sera is 42% (15/35).

To characterize the double band of about 14.5 kDa, we performed an immunoblotting inhibition study preincubating the sera of patients P32 and P20 with 100 µg/ml of profilin from pollens of *Artemisia vulgaris* (nArt v 4), *Phleum pratense* (rPhl p 12), *Phoenix dactylifera* (nPho d 2), *Olea europaea* (rOle e 2) and *Platanus acerifolia* (nPla a 8) (Figure 5). Only nPho d 2 achieved an almost complete IgE-binding inhibition to this 14.5 kDa band with both sera. In assay with P32 patient serum, a partial IgE-binding inhibition was observed with rOle e 2 (Figure 5). These results indicate that it is highly likely that the doublet-14.5 kDa-IgE binding protein corresponds with a leaf peach profilin that shares homology with date palm pollen profilin and not with the rest of profilins tested.

DISCUSSION

In adult patients with symptoms of rhinoconjunctivitis and asthma, an occupational disease should be ruled out (29). Only one (30) of review articles (31-36) on occupational respiratory allergy includes the peach tree as a source of allergens causing occupational respiratory diseases. In our case, symptoms with occupational exposure to peach trees suggested that they could be the cause, and the results of our study support this hypothesis.

The main sources of airborne plant allergens are pollens. Pollens from *Rosaceae* family have been rarely reported as a cause of occupational allergy. There are some cases among workers of strawberry (6) and rose (7) crops. The presence of allergens in *Rosaceae* fruit tree pollen has been documented by Berrens *et al.* (a Bet v 1 homologous protein in apple tree pollen) (37) and Marzban *et al.* (Mal d 1, Mal d 3 and their homologues in different *Rosaceae* fruit tree pollen) (38). However, the clinical significance of these findings for individuals with prolonged contact to high concentrations of fruit tree pollen by their profession needs to be clarified. Although exposure to *Rosaceae* fruit tree pollen is very common, we found only three papers reporting occupational allergy to that pollen, and two of them with artificial pollination. Teranishi *et al.* conducted an epidemiological study on pollinosis among Japanese pear (*Pyrus pyrifolia Nakai*) farmers and found positive skin tests in 5.4% of them, and specific IgE antibodies in their sera. All the patients had symptoms during the work period of artificial pollination (8). Jiang *et al.* have recently reported a case of occupational allergy due to peach tree pollen in a patient who worked facilitating artificial pollination. The authors identified and characterized an IgE-binding-20 kDa protein, homologue to glutathione s-transferase 16 from *Arabidopsis thaliana* showing cross-reactivity between pollens from different fruit trees of the same subfamily (9). Lara studied three workers of a peach orchard with rhinoconjunctivitis and asthma during flower thinning,

and pruning and harvesting fruit. The profile of IgE binding bands detected in pollen, leaf and branch extracts of peach tree was different for each of the patient serum studied. The molecular masses of the observed bands did not correspond with LTP or profilin ones. The author identified peach pollen as the cause of patient symptoms by SBCT. Unlike in our area, thinning is made with tree in bloom, removing flowers (in our area, fruits are removed). Patients showed *in vitro* sensitization to peach leaves that authors did not consider relevant (39). In our environment, the technique of artificial pollination is not used. Work in peach crops is usually done when trees are not in bloom. Pollination of cultivated varieties takes about 10-25 days (19). The characteristics of the plantations, which in the case of peach can be up to 800 trees per hectare, and the fact that the work is done manually, involve close contact of the worker and the aerial parts of the tree (40,41).

It has been shown that the leaves and non-pollen plant structures can trigger allergic respiratory diseases in workers of various crops such as melon (42) and asparagus (43). Among growers of fruit trees of *Rosaceae* family, some cases of occupational respiratory diseases have also been reported, specifically with leaves and other structures of almond tree (10), and peach tree leaves (11). Regarding the almond tree, Garrido Fernández *et al.* (10) studied a case of angioedema, urticaria and rhinoconjunctivitis after contact or proximity with cover fruit (cocoon) and almond leaves. Patient's serum reacted with a 9 kDa protein in cocoon and almond leaf extracts recognized by the anti-Pru p 3 monoclonal antibody. The authors concluded that the LTP present in the leaves of the almond tree and the almond mesocarp and epicarp acted as airborne and contact allergens.

The first publication of occupational asthma by peach tree appeared in 2004. Garcia *et al.* (11) studied a 27-year-old male farmer with a history of rhinoconjunctivitis and asthma when he worked in the peach grove when peach trees had leaves. The patient had a positive skin prick test with peach leaf extract and his serum mainly recognized a protein of about 14

kDa from the extract. IgE-binding to this band was inhibited by preincubation of patient serum with Pru p 3. The amount of Pru p 3 in the leaf peach tree and skin peach extracts was similar. The monoclonal anti-Pru p 3 recognized with similar affinity LTP from leaves and peach skin. The authors concluded that LTP from non-pollen structures like leaves can act as respiratory occupational allergen, and they stress the importance of these proteins as vegetable allergens. Subsequently, it has been reported a case of occupational asthma by peach tree leaves in a patient who worked in a laboratory analysis of fruit tree leaves and was sensitized to rPru p 3 (12).

In our study, the results of skin tests and specific IgE confirm that peach leaves, and branches exceptionally, can sensitize individuals occupationally exposed to peach trees. The SBCT with peach leaf extract confirms, in patients in whom it was performed, that this sensitization was the cause of symptoms. The SBCT was negative in patients P1 and P25. P25 had a low level of serum specific IgE to peach leaf extract and negative skin tests with this extract, and had only presented an asthma attack, being rhinoconjunctivitis its most common symptoms. P1 suffered from bronchial asthma and was not sensitized to peach leaf extract so this result allowed us to rule out that the extract was irritant. As a limitation in our study, we recognize that SBCT has not been done in all patients studied, in some cases by patient refusal and in others by working problems.

Most of our patients were sensitized to pollens (23: 62%). Thirteen patients were not sensitized to pollens and of these, 12 had sensitization to peach leaf extract and some other perennial allergens that we did not consider relevant. Interestingly, 6 of the patients in our total sample (16.2%) were monosensitized to peach leaves (Table 1). We emphasize the importance of our findings, as they have guided the diagnosis of these patients as occupational respiratory disease. We want to draw attention to the fact that cultivation of fruit trees of the *Rosaceae* family is widespread worldwide and, however, there have been

published very few cases of occupational respiratory disease. It may be an underdiagnosed condition since outdoor working coincides with periods of pollination, and symptoms can be attributed to exposure to common airborne allergens or considered non-allergic disease. Although there are individual cases published of respiratory allergy by handling (13) or exposure to peach in warehouses (14), none of our patients had symptoms in those situations.

The data obtained in our immunological studies suggest that several proteins in the leaf extract can sensitize individuals occupationally exposed to peach tree. Fifteen patients (42%) were sensitized to a protein homologous to Pru p 3 (LTP) present in the leaves of peach tree, and 12 patients were sensitized to a protein of about 14.5 kDa, which could correspond to a profilin with homology to the date palm pollen profilin (nPho d 2). LTP has been the panallergen associated with occupational asthma due to sensitization to peach leaves (11,12), exposure to peach (14) or exposure to other *Rosaceae* (10) or non *Rosaceae* (42,43) plants. In our case, we suggest that our patients could be sensitized to two LTP isoforms about 10 and 16 kDa from peach tree leaves.

No IgE-binding bands were detected in blotting assay with 11 sera from our sensitized patients. Some of the sera also detected other bands between 28 and 66 kDa and, interestingly, the serum of the patient P2 (with positive SBCT) recognized a single band of about 35 kDa. Further studies would be needed to characterize these proteins.

The analysis of the 8 patients in the sample (22%) who had contact urticaria during the thinning and harvesting of peaches (P3, P6, P16, P22, P23, P24, P29) provided some interesting findings. Respiratory and cutaneous symptoms started simultaneously in all of them except for P3 and P6 (cutaneous symptoms preceded respiratory ones). All tolerated the ingestion and manipulation of peach, and were sensitized to extracts of peach leaves (except P16), but prick test with Pru p 3 was negative in all, and only in P3 prick was positive with nPho d 2. In the study by García et al., they used as a control a peach worker with contact

urticaria with exposure to the leaves (without respiratory symptoms) who presented results similar to ours (11). Taking into account these findings, contact urticaria would be justified by the sensitization to peach leaves, but we do not know which allergen would be responsible.

CONCLUSIONS

When assessing patients with seasonal respiratory symptoms exposed to fruit trees we must question whether exposure to no-pollen tree structures may be the cause of the symptoms.

The peach tree acts as a source of airborne allergens in patients in our sample, which is the first series of cases of occupational allergy to peach tree. Most of our patients showed sensitization to the extract from the leaves and not to the branches.

Sera from 42% of patients revealed IgE-binding proteins matching with LTP, 34% with profilin, and 42% with other unidentified proteins

Additional studies are needed with larger samples and epidemiological studies to assess the real impact of this disease, especially in areas where this crop is so widespread.

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Legends

Figure 1.- SDS-PAGE immunoblotting with peach leaf extract without 2-mercaptoethanol (non-reducing conditions). Lanes 1-35: patient sera P1-P35; lane C: control serum (pool of sera from non-atopic subjects), lane M: molecular mass standard.

Figure 2.- SDS-PAGE immunoblotting with peach leaf extract with 2-mercaptoethanol (reducing conditions). Lanes 1-35: patient sera P1-P35; lane C: control serum (pool of sera from non-atopic subjects), lane M: molecular mass standard.

Figure 3.- SDS-PAGE immunoblotting with rabbit serum anti-Pru p 3. Lane 1: serum P4; lane 2: serum P11; lane C: control serum (pool of sera from non-atopic subjects); lane S: rabbit serum anti-Pru p 3; lane C₁: non-immunized rabbit serum; lane M: molecular mass standard. (-): without 2-mercaptoethanol; (+): with 2-mercaptoethanol

Figure 4.- SDS-PAGE immunoblotting inhibition with the sera of patients P4 and P18 and purified nPru p 3. Lanes 1-3: patient sera incubated with peach leaf extract (lane 1), nPru p 3 (lane 2) and ovalbumin (lane 3); lane C: control serum (pool of sera from non-atopic subjects); lane M: molecular mass standard.

Figure 5.- SDS-PAGE immunoblotting inhibition study with the sera of patients P32 and P20 and profilin from pollens of *Artemisia vulgaris* (nArt v 4), *Phoenix dactylifera* (nPho d 2), *Olea europaea* (rOle e 2) and *Platanus acerifolia* (nPla a 8). Lane C: control serum (pool of sera from non-atopic subjects). Lanes 1-6: patient sera preincubated with peach leaf extract (lane 1), nArt v 4 (lane 2), nPho d 2 (lane 3), rOle e 2 (lane 4), nPla a 8 (lane 5), ovalbumin (lane 6); lane M: molecular mass standard.

TABLE I.- Results of allergologic study

PATIENT	Leaf peach tree		LTP		Profilin			IgE binding bands kDa SDS-PAGE Immunoblotting					Prick pollens	Prick other allergenic sources	SBC
			rPru p 3		nPho d 2	nArt v 4	nPla a 8	without 2-mercaptoethanol (MET)		with MET					
	Prick	sIgE	Prick	sIgE	Prick	sIgE	sIgE	Bands 10-16 kDa		10 kDa	Double band 14.5 kDa	Other bands			
							10 kDa	16 KDa							
P1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P2	+	25.3	+	-	+	-	-	-	-	-	-	31	-	-	+
P3	+	10.4	-	-	+	-	-	-	-	-	-	-	-	M	+
P4	+	7.4	+	0.4	-	-	-	+	+	+	-	31	-	-	+
P5	+	-	+	-	+	-	-	-	-	-	-	-	G,O	M	ND
P6	+	0.7	-	-	-	-	-	-	-	-	-	-	G,O	-	ND
P7	+	19.2	+	2.5	-	-	-	+	+	+	-	-	-	-	ND
P8	+	0.4	-	-	-	-	-	-	-	-	-	-	-	Dog	ND
P9	-	-	-	-	-	-	-	-	-	-	-	39/49, 66	G	-	ND
P10	-	0.9	-	1	+	0.5	0.4	-	-	-	-	39/49	G,O,W	-	ND
P11	+	9.2	+	3.5	-	-	0.4	+	+	+	+	28, 39/49, 66	G,O	M,A,cat	ND
P12	-	1.3	-	-	-	-	-	-	-	-	-	-	-	-	ND
P13	+	10.1	+	6.6	-	-	-	+	+	+	-	-	-	-	ND
P14	+	-	-	-	-	-	-	-	-	-	-	-	-	M	ND
P15	+	58.3	-	-	-	-	-	-	-	-	-	-	G	-	ND
P16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND
P17	-	5.6	+	-	-	-	-	-	-	-	-	-	W	-	ND
P18	+	14.2	+	8.5	-	-	-	+	+	+	+	39/49, 66	G,P,C,W	-	ND
P19	+	3.2	-	-	+	7.3	4.0	-	+	-	+	-	G,O,W	-	ND
P20	-	2.8	-	-	+	1.9	0.7	-	+	-	+	28, 31, 66	G,P,C,W	-	ND
P21	-	-	-	ND	-	-	-	-	+	-	+	66	G,O	A	ND
P22	-	0.6	-	-	-	-	-	-	-	-	-	66	G,W	Cat	ND
P23	+	13.1	-	-	-	-	-	-	-	-	-	-	-	-	ND
P24	+	0.9	-	-	-	-	-	-	-	-	-	-	G,O	A	ND
P25	-	2.7	-	0.5	-	-	3.7	-	+	-	+	31,39/49	G,O	M,A	-
P26	+	1	-	-	-	-	-	-	-	-	-	-	-	M,cat	ND
P27	-	1.7	-	-	-	-	-	-	-	-	-	-	G	M,cat	ND
P28	-	0.4	-	-	-	-	-	-	+	+	+	66	G,W	-	ND
P29	-	-	-	-	-	-	-	-	-	-	+	39/49,66	W	-	ND
P30	+	2.6	-	-	-	-	-	-	-	-	-	-	-	M	ND
P31	-	1.5	-	-	-	1.8	0.7	-	+	-	+	66	G,W	M	ND

P32	-	1.7	-	-	-	-	0.4	-	+	-	+	66	G,O,W	A	ND
P33	+	0.9	+	-	+	-	-	-	+		+		-	M	ND
P34	+	7.8	+	6.8	+	16	9.3	+	+	+	-	-	G,W	-	+
P35	-	1.5	-	-	-	-	-	-	+	-	+	66	G,W	-	ND
P36	+	8.2	-	-	+	14.1	13.5	ND					G,O,W	-	ND
P37	+	6.8	-	-	+	12.1	6.7	ND					G,O,W	-	ND

A: *Alternaria sp.*; C: *Cupressus sp.* pollen; G: Grass pollens; LTP (Lipid transfer protein); M: Mites (house dust mites and/or storage mites); ND: not done; O: olive pollen; P: *Platanus sp.* pollen; SBC: specific bronchial challenge; sIgE: serum specific IgE; TU: *Tetranichus urticae*; W: weed pollens



