

Farm workers' occupational allergy to *Tetranychus urticae*: clinical and immunologic aspects

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A group of 46 farm workers (32 men), affected by a recurrent "occupational disease of undetermined origin", underwent an immunologic investigation with a *Tetranychus urticae* (TU) whole-body extract (TU-WBE) prepared in our laboratory. The patients suffered from seasonal attacks of rhinitis, during the summer and autumn periods, when working in open fields (30 subjects) or in greenhouse flower cultivation (16 subjects). In most patients, rhinitis was associated with bronchial asthma (16 subjects), urticaria (14 subjects), or both (three subjects). Allergic alveolitis or other common allergic diseases had been excluded, and a diagnosis of "occupational disease of undetermined origin" had been made before by other medical centers. Ten healthy farm workers and 10 atopic townsmen were chosen as control groups. An *in vivo* and *in vitro* diagnostic trial by skin prick testing (SPT) and serum specific IgE dosage with TU-WBE were done in all subjects. Thirty-six patients (78%) were found to be positive to both SPT and the IgE enzyme allergosorbent test (EAST), with a good correlation between IgE serum levels and cutaneous wheal size. Control groups did not show any reaction. The IgE-EAST homologous inhibition test was positive. The IgE-EAST cross-inhibition test excluded cross-reactivity between TU and *Dermatophagoides pteronyssinus*. The TU-exposure test was positive for the 36 patients with TU-WBE-specific IgE. Three patients who were negative for TU-WBE-specific IgE reacted to the TU-exposure test; in these patients, the scratch-chamber test (Finn chamber) with eggs and droppings from TU was positive. We suggest the possibility of a TU allergy also in those subjects in whom clinical symptoms can be attributed to other common allergens.

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Certain mite species of the phytophagous Tetranychidae family have been found to cause allergy. Among them, *Panonychus ulmi* (PU), a common parasite of fruit trees, has been found to cause occupational, allergic bronchial asthma (6, 9, 13). *Tetranychus macdanieli* (4) and *Tetranychus urticae* (TU) (1, 10) cause occupational respiratory symptoms in workers in both fields and greenhouses.

The *Panonychus* and *Tetranychus* species are macroscopic in size (0.5–1 mm), and they are classified in the order Prostigmata, family Tetranychidae, subfamily Tetranychinae (12). The Tetranychinae are phytophagous mites, found worldwide, which infest vegetables, ornamental flowers, other plants, and fruit trees. They are commonly called "spider mites" because of the web they produce. Because TU is the most common phytophagous mite in south-

ern Italy, farm workers living in this area are at risk of occupational, TU-caused allergic disease.

The present study reports the results of a selective allergic investigation in a group of farm workers who live in the Naples area, and who are affected by recurrent occupational episodes of rhinitis associated, in some of them, with bronchial asthma or urticaria. The patients were selected by other medical centers, in which definite occupational diseases, hypersensitivity pneumonia (11), and allergic diseases caused by common allergens had been excluded and a diagnosis of "occupational disease of undetermined origin" had been made. Since these symptoms strongly suggest allergic occupational disease, the patients were referred to us for investigation of the possible occurrence of TU-caused allergic disease, by *in vivo* and *in vitro* tests with a TU

whole-body extract (TU-WBE) prepared in our laboratory.

Material and methods

Patients and controls

The patients consisted of 46 farm workers (32 men) referred to us as outpatients over a 3-year period. Ten healthy farm workers and 10 atopic townsmen were chosen as control groups.

Upon examination, the clinical history of each patient was recorded by a standard questionnaire. The presence of atopic subjects in the family and whether the patients had suffered from atopic diseases (allergic asthma, rhinitis, or both, and cow's milk intolerance) in the past were investigated. Information on the relationship between the total farm-work activity and onset time of the present clinical situation was obtained. Most of our patients (34 subjects) could trace their symptoms to contact with TU.

All patients suffered from seasonal sneezing attacks, nasal itching, and aqueous rhinorrhea with variable nasal blockage during the summer-autumn period, while working in open fields (30 subjects) or in greenhouse flower cultivation (16 subjects). In most patients, nasal symptoms were associated with acute dyspnea and dry cough (16 subjects), itchy wheals localized on uncovered skin of arm and face (14 subjects), or both symptoms (three subjects). All patients had been suffering for more than 2 years, and symptoms appeared only while patients were working. These symptoms lasted no longer than 2 h after finishing work. Four patients sporadically experienced a late respiratory complaint which lasted 12–24 h after the immediate reaction.

Among the patients, 91% had family members with a history of allergy, hay fever, and respiratory problems. Five of these patients had suffered from allergic diseases such as cow's milk protein intolerance (one subject), isolated rhinitis (three subjects), or respiratory complaints (one subject) caused by house-dust allergy during infancy.

The 46 patients were selected by final diagnosis of "occupational disease of undetermined origin" from 440 farm workers who had received a diagnosis of nontraumatic, allergic or nonallergic, occupational disease within the last 3 years at occupational medicine centers. Such centers usually examine about 5000 farm workers yearly.

The 46 patients we studied came from 40 different farms, which yearly hired altogether about 800 farm workers. Among the patients studied, the duration of the activity as farm worker before the onset of the occupational symptoms is shown in Fig. 1.

The study involved two control groups. Ten healthy TU-exposed farm workers and 10 atopic

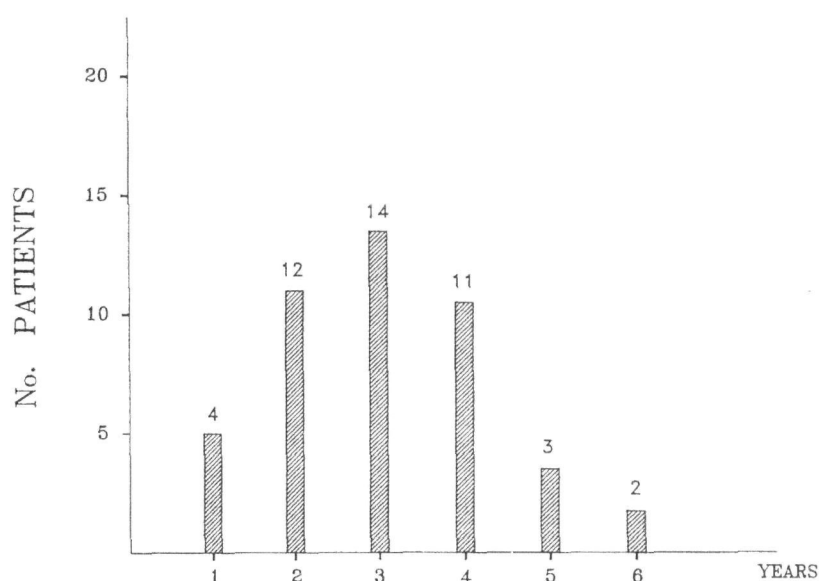


Fig. 1. Years of activity as farm worker before onset of symptoms.

townsmen affected by rhinitis caused by *Dermatophagoides pteronyssinus* (Dp) were selected. The average age of the investigated subjects was 29 years, and there was no statistically significant difference in age between the group of patients and the controls.

Diagnostic trial

Patients underwent a diagnostic allergic trial with *in vivo* skin prick testing (SPT), in duplicate for each extract, with inhalant (*Dermatophagoides* and storage mites, pollens, and molds) and food allergens (DHS, Bayropharm, Milan, Italy, as well as the TU-WBE prepared in our laboratory. A TU-WBE, serum specific IgE dosage was administered in all patients. Finally, a complete clinical examination and measurement of the peak expiratory flow rate (PEFR) were done before and after exposure to the putative allergen (see below).

Preparation of TU cultures and extracts for diagnostic use

The mites (TU; 40 males, 150 females) were isolated, identified by stereomicroscopic examination, and transferred on fresh bean leaves into Petri dishes at 28°C and 20–40% humidity in a sterile, laminar air-flow chamber. The bean leaves were collected from seed-sprouting plants cultivated in soil free of insects, molds, and mites. Mites were cultured in a sterile, laminar air-flow chamber at 26°C, 60–70% humidity, and 600 lx.

Every 4 days, two leaves were restocked for each Petri dish. Every 2 weeks, most of the mites (80–90%) were gently collected, lyophilized, and stored at –80°C in sterile tubes. The mites left on the leaves were brushed onto virgin leaves in a new Petri dish.

Whole TU body extract was prepared by incubation (overhead rotation) of lyophilized mite at 4°C

in a 0.01 M NH_4HCO_3 , pH 8.6, solution for 4 h (20 mg lyoph./ml). Samples (10 ml) of TU homogenate were centrifuged for 10 min at 4500 g, and the supernatant was carefully removed. The supernatant was then concentrated on an Amicon YM5 (Amicon, Denver, CO, USA) (molecular cutoff at 5000) and finally dialyzed against PBS. The extract was aliquoted and stored at -20°C until used. The protein concentration was 1.1 g/l.

In vivo study

For SPT, 1 ml of supernatant, sterilized by Millipore filtration, was diluted at 1:30 in a 0.1% HSA and 0.5% phenol, saline solution.

SPT results were recorded after 15 min and compared with HCl-histamine (10 mg/ml) as standard positive reference and the diluent as a control. SPT was considered to be positive when it showed a wheal with a mean diameter (the mean of the longest and the midpoint orthogonal diameters) (2) at least 3 mm larger than the negative reference.

In vitro study

Enzyme allergosorbent test (EAST). IgE-binding components in TU extracts were investigated by EAST with extract bound to cyanogen bromide-activated cellulose disks with 1 ml of extract diluted to 2.0 ml with 0.1 M NaHCO_3 (pH 8.4)/10 activated disks. IgE-EAST was done according to the manufacturer's instructions (Phadezym RAST-Pharmacia, Uppsala, Sweden). All patients and control subjects were tested. IgE titers were expressed in absorbance values at 420 nm. A value at least 2 SD above the higher mean value of the control groups was considered to be IgE-EAST positive.

IgE-EAST inhibition. For investigation of the reliability of the TU IgE-EAST and the presence of some cross-reactivity between TU and Dp, IgE-EAST homologous inhibition and cross-inhibition experiments were done. A pool of 26 patient sera containing the higher values of TU-specific IgE was used to perform the homologous inhibition. A volume of 50 μl of pool serum was added to 100 μl of RAST buffer solution containing increasing amounts of TU extract dry weight material (0.1, 1.0, 10, 100, and 1000 $\mu\text{g}/25 \mu\text{l}$ serum). After a 3-h incubation at room temperature, residual specific IgE was measured by the IgE-EAST assay.

The IgE-EAST-cross-inhibition experiment was performed as follows: the same pool serum was incubated with increasing amounts of standardized, purified Dp extract (Pharmacia, Uppsala, Sweden) 100000 biologic units (BU)/ml.

IgE-EAST inhibition experiments were also done

with a pool of five patient sera containing high titers of Dp-specific IgE, by incubation with the same inhibitors (TU extract and Dp).

Exposure test to TU

All patients were submitted to an exposure test in a pesticide-free, oleander greenhouse infested by TU. The exposure time was 2 h, and all patients were asked to behave as usual in their normal work. Patients were randomly assigned to each of the authors, who followed up their clinical situations and PEFR before, during, and after the exposure period at 30-min intervals for as long as 6 h. A $>20\%$ fall of PEFR was considered to be positive.

Statistics

Statistical comparisons were made between SPT results and IgE-EAST values with Pearson's correlation. Data are presented as the mean $\pm$ SD of the mean. Differences at the 0.05 level or less were considered to be significant (5).

Results

SPT and clinical aspects

Among the patients, 36/46 (78.2%) reacted to TU extract. Wheal mean diameter ranged between 5.4 and 18 mm (mean = 10.5 ± 3.1 mm). No positive reactions were observed in the control groups. Among TU-positive patients, an additional positivity to Dp in two patients who had suffered from allergic rhinitis in infancy was found.

All patients studied suffered from rhinitis (nasal obstruction and itching, rhinorrhea, and sneezing), while asthma (reversible airways obstruction associated with dyspnea, cough, and wheezing) was present in a similar percentage among the TU-SPT-positive (16/36 = 44%) and negative (3/10 = 30%) subjects. All patients affected by urticaria associated with rhinitis (14/46) or with rhinitis plus bronchial asthma (3/46) were TU-SPT-positive. Among the TU-SPT-negative subjects, there was a higher prevalence of isolated rhinitis (7/10 = 70%).

All symptoms were strictly related to the patients' working activity.

IgE-EAST and IgE-EAST inhibition

All the TU-SPT-positive patients had a TU-specific serum IgE concentration at least 2 SD above the mean value (0.18 ± 0.06) of controls; no statistically significant differences in serum TU-specific IgE were found among the TU-SPT nonreactive patients and the control groups (Fig. 2). In our patients' group, a good individual correlation between the mean diam-

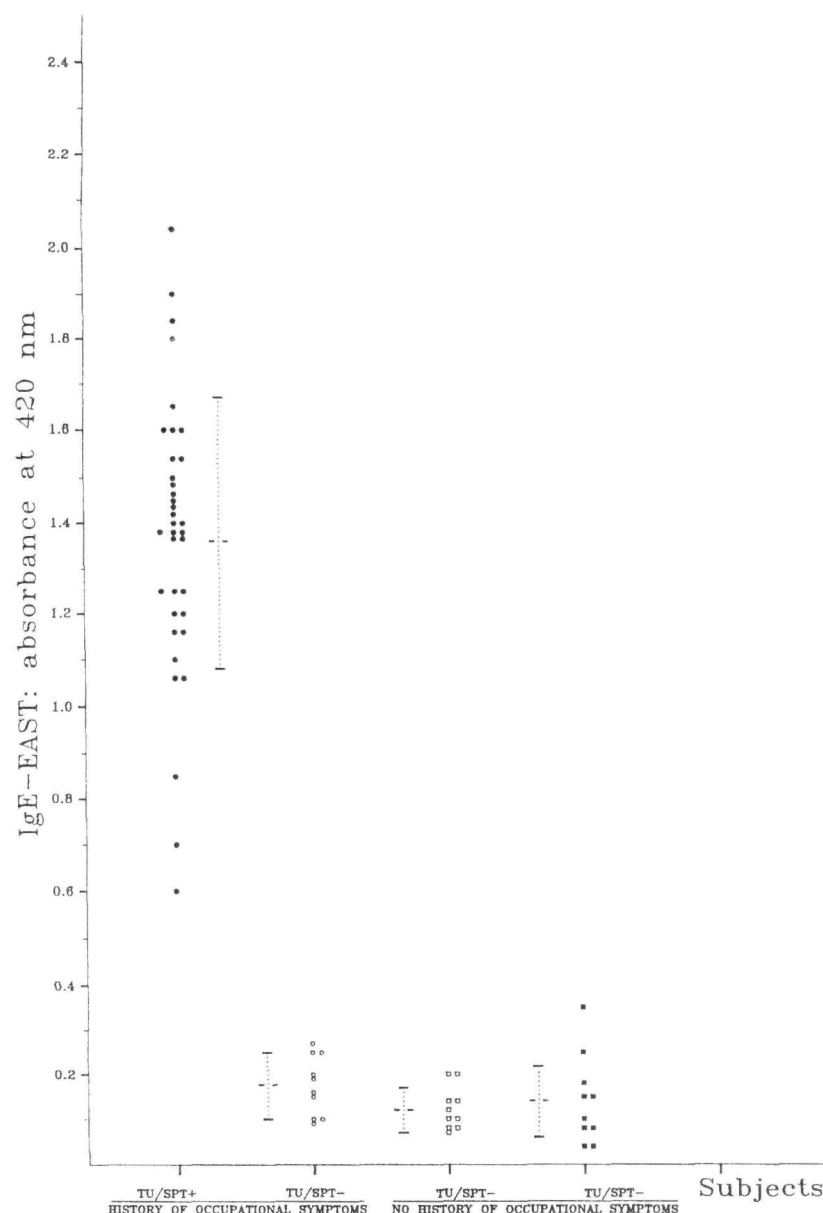


Fig. 2. TU-specific IgE serum levels grouped by clinical history of TU-sensitivity and results of SPT with TU. ●, ○: examined patients; □: atopic, non-TU-exposed subjects; ■: TU-exposed healthy farm workers.

eter of TU-induced wheals and titers of TU-specific IgE was found ($r = 0.81$; $P < 0.001$) (Fig. 3).

TU-specific IgE-EAST homologous inhibition experiments showed a 100% reduction of the specific absorbance, as compared with the 20% obtained with Dp extract in the IgE-EAST cross-inhibition assay (Fig. 4). The same experiment, performed with Dp-sensitive subject pool serum, showed a homologous inhibition of 90% and a cross-inhibition (preincubation with TU extract) of 20%. The low titer of Dp-specific IgE present in pool serum from TU-sensitive farm workers was slightly modified after preincubation with TU antigens (Fig. 5).

Exposure test

All the patients with positive TU-SPT and serum IgE ($n = 36$) quickly exhibited the onset of respiratory and cutaneous symptoms when reexposed to TU in the greenhouse (8–45 min; mean: 18 min). All of them showed rhinitis, either isolated (3/36) or

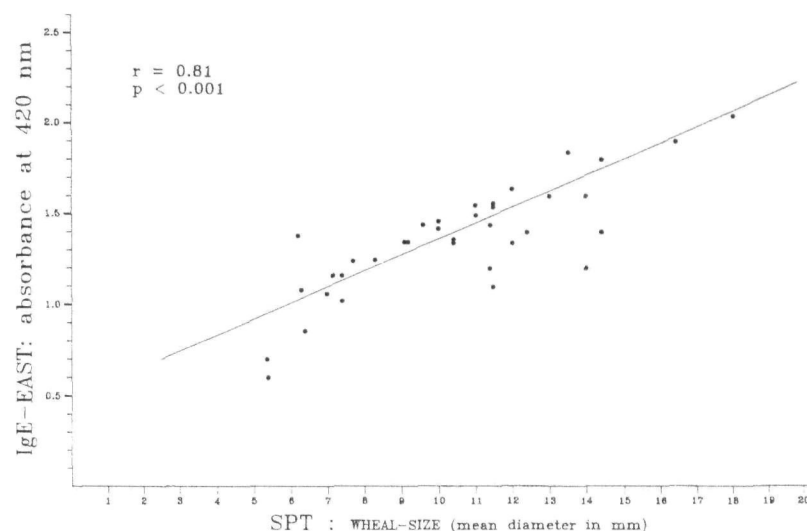


Fig. 3. IgE-EAST/SPT relationship in 36 patients with TU-positive SPT and serum specific IgE. TU-specific IgE values are on ordinate. On abscissa are mean diameters of TU-caused cutaneous wheals. Regression line is plotted, and correlation coefficient and probability are listed.

associated with bronchial asthma (16/36), urticaria (14/36), or both symptoms (3/36).

In the asthmatic patients, a >20% reduction of PEFR below preexposure values was observed within a range of 30–90 min after the beginning of the challenge. A complete normalization of asthmatic complaints (two normal consecutive determinations of PEFR) was observed, for the longest extent, 2 h after the appearance of asthmatic symptoms. No late-phase reactions (nasal or bronchial) were mentioned by any patient.

The cutaneous itchy wheals that we observed in 19 patients appeared exclusively on unabraded and uncovered skin of arms and face, resembling the clinical picture of “contact urticaria” (8).

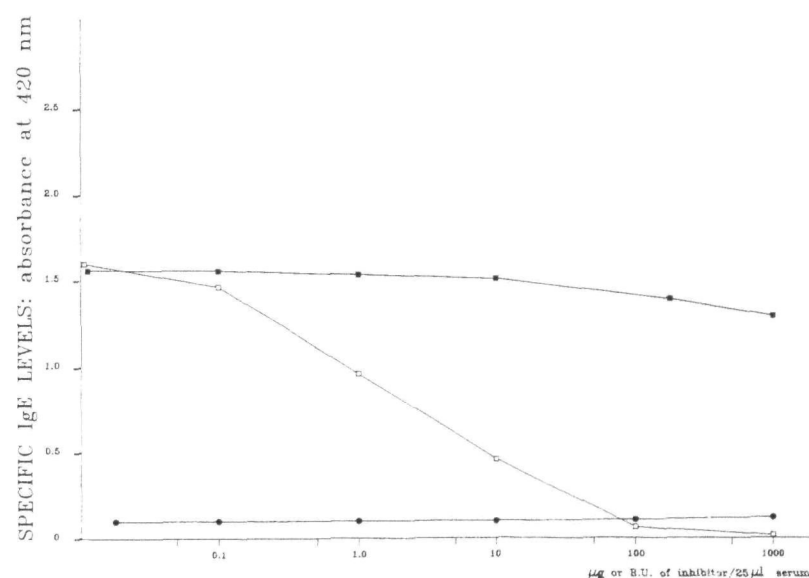


Fig. 4. IgE-EAST inhibition of Dp and TU extracts with TU-allergen disk. (□) Homologous- or (■) cross-inhibition experiments with pool of 26 patient sera containing high titers of TU-specific IgE. Homologous inhibition experiments using pool sera of (●) five atopic subjects containing high titers of Dp-specific IgE. Specific IgE levels are expressed as absorbance at 420 nm.

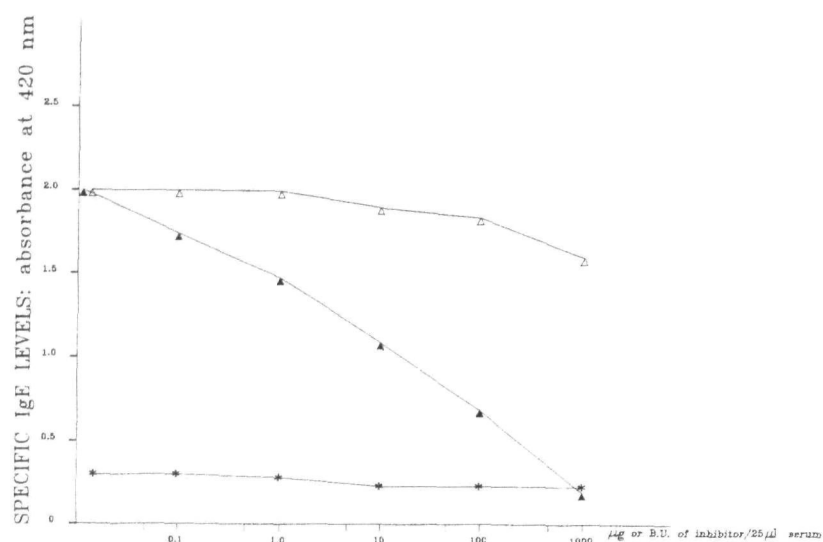


Fig. 5. IgE-EAST inhibition of Dp and TU extracts with Dp-allergen disk. (▲) Homologous- or (△) cross-inhibition experiments with pool serum of five subjects' sera containing high titers of Dp-specific IgE. (*) Cross-inhibition experiment with pool of 26 patient sera containing high titers of TU-specific IgE. Specific IgE levels are expressed as absorbance at 420 nm.

Only 3/10 patients with negative TU-SPT and serum IgE developed nasal and ocular itching, sneezing, and rhinorrhea. These three patients showed a positive scratch-chamber test (Finn chamber) reaction (7) to crushed bean leaves preinfested with mite. Adult TU mites had previously been removed from the leaves, on which their eggs and droppings had been left, under stereomicroscopic control.

All the symptoms induced by the exposure test were easily controlled by a single dose of oral antihistamine (terfenadine 120 mg) or inhaled β_2 -agonist bronchodilator (salbutamol 0.2–0.4 mg).

Discussion

Our results confirm that TU is a source of occupational allergen. Most of the patients studied (80%) presented the onset of occupational TU-caused allergic syndrome after a 2–4-year period of exposure to the allergen.

Rhinitis is the main symptom of a more complex, TU-caused, allergic syndrome with variable association with bronchial asthma and contact urticaria. All these symptoms are strictly related to the occupational activity as confirmed by exposure test in TU-infested greenhouses.

Among the patients studied, three of the subjects nonreactive to TU-WBE showed a positive clinical challenge in TU-infested greenhouses. Their positivity to the scratch-chamber test (Finn chamber) led us to assume they were allergic to segregated allergens contained in TU droppings and eggs.

Seven patients did not react to TU-SPT, IgE-EAST, and the exposure test. In these cases the recurrent occupational symptoms may have been due to an allergic response to uninvestigated aller-

gens or to a nonallergic adverse reaction to pesticides (3) or other chemical agents widely used in intensive farming, and not present in our experience.

Regarding immunologic aspects, there is an evident correlation between *in vivo* (SPT) investigation and *in vitro* (IgE-EAST) investigation with our TU-WBE. Our results confirm the absence of any cross-reactivity between Dp and TU (10), while a partial *in vitro* cross-reactivity has been reported by Kroidl et al. (6) between Dp and PU, another phytophagous mite. This suggests the existence of individual antigenic profiles among nonpyroglyphic mites.

The patients studied presented an isolated allergic reactivity to TU, according to selection criteria. We cannot exclude that in other farm workers affected by occupational allergic symptoms the TU hypersensitivity may exist in association with hypersensitivity to other common occupational allergens. Therefore, in order to optimize the therapeutic approach, we suggest checking the TU-induced allergy in all patients with specific occupational risk.

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