

Specific bronchial challenge tests. The specific bronchial challenge test comprises the inhalation of the suspected agent initially at low and then at progressively increasing doses, without exceeding the irritant concentration. In the clinical context, this test is used for confirmation of the diagnosis of OA, but it cannot be applied *en masse* to all the participants in epidemiological studies. Even so, in certain cases it must be used for confirmation of the diagnosis in workers in whom the screening methods have suggested the presence of OA.

The specific bronchial challenge tests serve, as does the serial monitoring of the PEF, for objectively demonstrating that a relation exists between the bronchial asthma symptoms of the patient and the exposure to an environmental agent present in the work environment, and represent the most reliable test in the diagnosis of OA.

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Epidemiological study of occupational *Tetranychus urticae* allergy among greenhouse workers

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Phytophagous mites usually known as "red spiders" or "red spider mites" are common parasites of agricultural and ornamental plants and of fruit trees. One of such red spider mites is *Tetranychus urticae*, a macroscopically visible mite (0.3 - 0.5 mm in length) belonging to the Order *Acari*, Suborder *Pros-tigmata*, Family *Tetranychidae*. Its distribution is universal, particularly in warm humid climates, and it is considered to be the most frequent and harmful *Tetranychid* in herbaceous plants, as it may achieve optimal development and reproduction on over 150 economically important vegetable species¹.

Agriculture represents by far the most important occupation in our Health Care catchment area, and particularly so the greenhouse cultivation of carnations, which because of its particular

temperature and humidity characteristics often suffers infestation by *T. urticae*. It is calculated that *ca.* 13,000 individuals are partially or fully dedicated to this activity, and this has caused a high number of greenhouse workers to be among our patients. These patients had been observed to meet the criteria for allergic disease, yet in many cases no sensitization to the commonly encountered allergens could be demonstrated.

After becoming aware of a number of descriptions of isolated cases of *T. urticae* hypersensitivity^{2,4}, we initiated a study on a sample of greenhouse workers consulting because of allergic conditions, with the support of the SEAIC Foundation⁵. It was seen that *T. urticae* evidenced a high sensitizing capacity (66%) and, in our experience, both the skin prick tests and the RAST offered high diagnostic cost-effectiveness⁶.

Further studies have corroborated the importance of *T. urticae* as an occupational allergen among open-field agricultural workers⁷, as well as its ability to induce sensitization either by itself or in association to other *Tetranychids*⁸. In some cases, a Type IV hypersensitivity mechanism has been demonstrated⁹.

This allergen has been characterised with the help of immunodetection studies and a relevant protein band in the 19-kDa range has been observed, with no appreciable cross reactivity to *Dermatophagoides pteronyssinus*¹⁰. The sensitizing capacity of *T. urticae* in relation to the parasited agricultural cultivation has also been assessed in an investigation supported by a grant from the SEAIC Foundation; the preliminary results of this study were presented at Rhodes¹¹.

T. urticae is thus a newly-characterised allergen and, although the studies carried out would seem to establish it as a highly-sensitizing occupational allergen, all these studies have been carried out on samples of patients with symptoms suggestive of occupational allergy. There are as yet no epidemiological data available on the true prevalence of *T. urticae* sensitization among exposed workers. For this reason, a cross-sectional epidemiological study was designed for assessing the importance of *T. urticae* as the cause of occupational disease among greenhouse workers, again with the support of the SEAIC Foundation.

The aims of this study have been: (1) to assess the prevalence of *T. urticae* sensitization and *T.*

urticae-induced occupational asthma in a representative sample of greenhouse workers, and (2) to identify risk factors among the workers leading to a prediction of future development of *T. urticae* sensitization: age, gender, atopy, type of cultivation, continuous or sporadic exposure and latency period for sensitization.

MATERIAL AND METHODS

Selection of the sample. A field study was carried out on two co-operative enterprises in the Los Palacios municipality (Seville), which together occupy about 1,500 greenhouse workers in carnation cultivations and a further 300 greenhouse workers dedicated to garden vegetables. The actual study population comprised 246 workers, selected at random with the following inclusion criteria: at least one year of exposure, and belonging to a complete work nucleus.

A total of 152 atopics and 75 non-allergic patients from our Allergy Unit, none of them with agricultural activity, were included as controls.

Clinical questionnaire. The questionnaire was administered in all cases and controls by the same person, and collected the following data: personal demography (age, gender); personal atopy history (previous positive prick tests to airborne allergens); symptomatology prior to working in the greenhouse; apparition or worsening of the clinical symptoms in the greenhouse; recognition of *T. urticae* in the work environment; worsening of the condition in relation to *T. urticae* and relation exclusively with it; duration of exposure (years of work in the greenhouse); latency period (difference between the exposure period and the initiation of symptoms); type of exposure (continuous or temporal, less than six months in the year), and actual type of cultivation with which the worker had relation (carnations or garden vegetables).

Skin tests

(a) *T. urticae*. The allergenic extract, with documented diagnostic value¹⁰, was prepared from carnation stem leaves that were densely parasited by *T. urticae*. The leaves were stirred in PBS (100 mg/ml) for 12 hours, and the extract was filtered and lyophilised. The concentration was homologa-

ted with the help of a reference extract by means of RAST inhibition, the resulting final concentration being 2 mg/ml. The prick tests were performed in duplicate, according to the EAACI recommendations for epidemiological studies¹².

(b) Airborne allergen panel. This panel comprised commercially acquired mites (*D. pteronyssinus*, *D. farinae*), *Alternaria*, epithelia (cat and dog), pollen (grasses, *Olea*, *Chenopodium*) extracts from ALK-Abelló, SA (Madrid, Spain). The tests were carried out with the usual skin prick technique.

The tests were read after 15 minutes, and the response was considered to be positive when the mean diameter of the wheal was greater than 3 mm (with subtraction of the diameter of the wheal eventually induced by the negative control tests).

Specific IgE assessment

The specific IgE was quantitated by the RAST technique, using the same extract as in the skin prick tests. This was performed as a diagnostic support in the following cases:

(a) In all greenhouse workers reporting occupational worsening, as well as in all those in whom the prick test results were positive or doubtful. It was also carried out as a control in 25 workers with no occupation-related clinical manifestations and with negative prick tests.

(b) In the atopic and non-atopic not exposed control subjects, if they evidenced positive prick tests to *T. urticae*.

The specific IgE assessment was considered to be positive at Class 1 or higher levels; the Class classification was as follows: RAST Class 0, <0.35; RAST Class 1, 0.35 - 0.7; RAST Class 2, 0.7 - 3.5; RAST Class 3, 3.55 - 17.5, and RAST Class 4, >17.5 PRU/ml.

Definitions

Considering that *T. urticae* is a common greenhouse pest, subjects were considered to be "exposed and sensitized" to *T. urticae* when the prick test was positive in a greenhouse worker, and "exposed and allergic" to *T. urticae* when, besides the positive prick test, there were clinical manifestations suggestive of allergic disease (apparition or worsening of the symptoms while in the greenhouse).

Statistics

The SPSS version 2.0 statistical package was used in a Pentium MMX 166 personal computer. Parametric

tests were used for samples fulfilling normal distribution criteria and non-parametric tests when this condition was not met. The χ^2 test was used for comparing qualitative variables, with Yates' correction when the number of effectives was less than 5 in any of the conditions.

RESULTS

(A) Greenhouse workers

The group of 246 greenhouse workers comprised 142 males and 104 females (58% and 42%, respectively), with a mean age of 33.16 years (SD, 13.01 years). Eighty workers (32%) had a personal history of atopy.

Clinical data. Almost all the workers (93%) recognised *T. urticae* as a habitual pest in their greenhouses. Clinical symptoms suggestive of allergic disease were reported by 28.5% of the workers in relation to their work at the greenhouse, and 81% of them had begun to experience these symptoms after starting to work in this environment. Conversely, 71.5% of the workers remained asymptomatic in their work environment.

Skin tests to T. urticae. Dermographism with negative specific IgE was observed in five out of the 146 workers. These subjects were therefore excluded from the study, and the data actually correspond to a study population of 241 workers, with the following results: not sensitized (negative prick test to *T. urticae*), 180/241 workers (75% of the sample); sensitized (positive prick test with *T. urticae*), 61 workers (25% of the sample). Among the latter, 16 were monosensitive to *T. urticae* and the remainder reacted to further airborne allergens (mites 29, pollen 27, epithelia 6, *Alternaria* 1).

Specific IgE. A specific IgE quantitation was carried out in the following greenhouse workers:

- In 60 out of 61 workers with positive prick test to *T. urticae*: positive in 29 cases and negative in 31, with the following class distribution: Class 0, 32; Class 1, 12; Class 2, 10; Class 3, 6; Class 4, 1.
- In 6 workers with inconclusive prick test: all these workers had a Class 0 RAST result.
- In 24 workers reporting occupation-related worsening of their complaint despite negative prick tests with *T. urticae*: negative RAST in all cases.
- In 25 workers with no occupation-related cli-

Table I. Correlation between the results of the skin prick tests to *T. urticae* and the clinical data

Clinical symptoms while in the greenhouse	(-) Prick test to <i>T. urticae</i>	(+) Prick test to <i>T. urticae</i>	Total
Yes	24	45	69
No	156	16	172
Total	180	61	241

nical symptoms and with negative prick tests: Class 0 RAST results in all of them.

Relationship between the clinical data and the results of the diagnostic techniques (Table I). Out of the 241 workers, 45 (19% of the sample) reported clinical symptomatology and had positive prick tests to *T. urticae*, and they were therefore considered to be allergic to *T. urticae* (ATU). The remainder of the workers (81%) were classed as "not allergic to *T. urticae*" (NATU). There were no significant age or gender differences between the two groups. Sixteen workers (7%) evidenced subclinical sensitization (absence of symptoms despite positive prick tests to *T. urticae*); the specific IgE quantitation was negative in all 16 cases.

Among the 69 workers reporting symptoms when in the greenhouse, sensitization to *T. urticae* was detected in 45 ($p < 0.0001$). This means that, in our environment, a greenhouse worker reporting allergic symptoms has a 65.5% probability of having a positive prick test to *T. urticae*.

Among the 45 exposed workers considered to be allergic to *T. urticae*, there were symptomatology compatible with rhinoconjunctivitis in 43 (96%), with asthma in 17 (38%) and with urticaria in 12 (27%).

The specific IgE quantitation was positive in 24 of the 45 workers with positive prick tests, with compatible clinical manifestations in all but two of the cases. In the cases in which it was performed, the sensitivity of the specific IgE assessment was only 53% of that of the prick test, although its specificity was 97%. The positive predictive value was 92%, and the negative predictive value 76%.

In the considerations next following, the analysis of the results is restricted to the 45 workers considered to be allergic to *T. urticae* (ATU).

Table II. Correlation between *T. urticae* allergy and atopy

Atopy	NATU	ATU
Yes	44 (55%)	36 (45%)
No	152 (94%)	9 (6%)

NATU: not allergic to *T. urticae*; ATU: allergic to *T. urticae*.

Relationship to atopy. A greater percentage of ATU cases was found among the atopic workers than among the non-atopic ones ($p < 0.0001$) (Table II).

*Worsening upon exposure to *T. urticae*.* In the clinical questionnaire, 42 workers reported observing worsening of their symptoms in relation with the exposure to *T. urticae*, and in 15 cases symptoms occurred exclusively when exposed. When these data are considered in relation to *T. urticae* allergy, a statistically highly significant ($p < 0.0001$) is observed between subjective worsening upon exposure to *T. urticae* and allergy to this mite (Table III).

Form of exposure. Ninety-four per cent of the surveyed workers had continuous occupation, and only 15 were temporary employees. There was no statistically significant association between form of exposure and *T. urticae* allergy.

Type of cultivated plants. In most cases (53%) carnations were the only plants cultivated in the greenhouses; there was mixed cultivation in 32% of the cases, and exclusive vegetable cultivation in 15%. There were no significant differences between carnation and vegetable workers as to the proportion of subjects allergic to *T. urticae*.

Duration of exposure. The mean duration of the occupational exposure was 6.57 years (SD, 4.17 years). The mean duration of exposure was 7.24

Table III. Correlation between *T. urticae* allergy and worsening upon exposure to *T. urticae*

Worsening upon exposure to <i>T. urticae</i>	NATU	ATU	Total
Yes	14	28	42
No	182	17	199
Total	196	45	241

NATU: not allergic to *T. urticae*; ATU: allergic to *T. urticae*.

Table IV. Correlation between occupational exposure and *T. urticae* sensitization

	Positive prick test to <i>T. urticae</i>	Negative prick test to <i>T. urticae</i>	Total
Exposed (greenhouse)	61	180	241
Not exposed (controls)	19	208	227
Total	80	388	568

years in the ATU cases and 6.42 years in the NATU ones, the difference being significant ($p < 0.05$). In the subjects with subclinical sensitization, the mean duration of exposure was 8 years.

Latency period. This was calculated for each group of symptoms only in the ATU subjects who had not had symptoms prior to working in the greenhouse. The latency period was 3.18 years for rhinoconjunctivitis, 4.73 years for asthma and 3.4 years for urticaria. The differences were non-significant.

(B) Non-exposed non-atopic controls

The sample population of 75 non-atopic controls comprised 20 males and 55 females, with a mean age of 36.4 years. The skin prick tests to *T. urticae* were negative in all cases.

(C) Non-exposed atopic controls

The sample population of 152 atopic controls comprised 71 males and 81 females, with a mean age of 26.39 years.

Skin tests and specific IgE to *T. urticae*. The skin prick tests to *T. urticae* were negative in 133 atopics (87.5%) and positive in 19 (12.5%), although with a wheal size in the latter which was significantly smaller than that seen among the greenhouse workers. The specific IgE to *T. urticae* was negative in all the subjects in this control group.

Among the 19 positive cases, 16 were sensitized to pollen, 5 to epithelia, 3 to moulds and 11 to mites. Out of 68 subjects with mite allergy in this particular control group, there was only one positive result among these eleven.

(D) Statistical study

In the analysis of the skin sensitization to *T. urticae* in the overall sample populations of exposed and control individuals, it was found that *T. urticae* sensitization is related to occupational exposure: the pro-

bability of becoming sensitized is 3.7 times higher among exposed workers ($p < 0.0001$). The overall prevalence was 17% (Table IV).

DISCUSSION

The existence of a considerable percentage of incapacitating occupational disease among greenhouse workers might be explained by the exposure to a large number of potentially noxious factors ranging from the environmental climatic conditions (temperature and humidity) within the greenhouses to the exposure to irritant substances (insecticides) and to potential allergens.

As in all conditions related to occupational allergens, occupational, social and economic implications of great importance coexist in the *T. urticae*-induced complaints; these implications are important for a considerable number of families/households that derive their income from intensive greenhouse cultures. As *Tetranychus urticae* has not been assessed and considered as a possible occupational allergen until recent years, the aim of the present work has been to compile data leading to a better understanding of this occupational condition, which may then facilitate individualised therapeutic and prophylactic decision making. In summary, our observations have been as follows:

- In our environment, most greenhouse workers are dedicated to the cultivation of carnations and are continuously occupied in it. Those working exclusively with vegetables or working only temporarily constitute a minority.

- As *T. urticae* is a macroscopically visible mite, it was easily identified by 93% of the workers when it was present in the greenhouse. This will facilitate carrying out the available prophylactic or therapeutic measures.

- 28.5% of the greenhouse workers report

occupational symptoms, and over one half (ca. 60%) describe worsening of their symptoms upon exposure to *T. urticae*.

- One fourth of the greenhouse workers are sensitized to *T. urticae*.

- The prevalence of occupational allergy to *T. urticae* has been 19% among greenhouse workers.

- In our environment, a worker describing allergic symptoms when in the greenhouse will have a 65.5% probability of a positive skin prick test to *T. urticae*. This means that approximately two out of every three workers reporting symptoms while in the greenhouse will have positive skin tests to this mite. Regarding the remainder of the cases, it should be remembered that other causes exist within the greenhouses that might elicit occupationally related symptoms.

- Subclinical sensitization to *T. urticae* was detected in 7% of the present sample of greenhouse workers.

- As in the case of other high-molecular-weight occupational allergens, atopy represents a significant predisposing condition for sensitization to *T. urticae*: 45% of the atopic subjects working in a greenhouse become allergic to this mite, while this will happen in only 6% of the non-atopic workers.

- Among the subjects reporting worsening of their symptoms upon exposure to *T. urticae*, the probability of being allergic to this mite is 63%.

- In the present study no significant differences were detected regarding a greater sensitizing power of continuous or temporary exposure to this allergen.

- The actual type of cultivation does not appear to significantly influence the predisposition to sensitization.

- The mean duration of the exposure was greater among patients allergic to *T. urticae* as compared to the remainder of the workers. No differences were observed in the latency periods for the various clinical manifestations.

- The results of the skin prick tests to *T. urticae* were negative in all the 75 non-atopic control subjects.

- Among the atopic control subjects, positive skin prick tests were observed in 19, with a significantly smaller wheal size and with negative specific IgE. This observation might be explained by the fact that these subjects live in a rural environment, by their having ornamental plants in their

household environments, or by cross reactivity to other allergens.

- In the present study, the specific IgE assessment was less sensitive than the skin prick tests.

In conclusion, it should be remembered that to the cultivations which have been traditionally carried out in greenhouses (flowers, leaf vegetables) further ones are being added which yield considerable benefits when carried out under these conditions. It would appear to be evident that in years to come this particular form of agricultural development will be extended to other crops that are currently developed as open-field ones. Furthermore, it should not be forgotten that a number of open-field crops (particularly fruit trees, cotton and grapes) may also be parasited by *T. urticae* as an agricultural pest.

As a significant pathogenic role of *Tetranychus urticae* has been confirmed, particularly intense and early eradication procedures are required and warranted, as well as an individualised approach to the sensitized individuals.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of the Sociedad Española de Alergología e Inmunología Clínica (SEAIC) Foundation.

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SECOND SESSION: PRACTICAL USEFULNESS OF TRYPTASE QUANTIFICATION IN THE DIAGNOSIS OF ALLERGIC DISEASES

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Quantification of serum tryptase in anaphylaxis patients

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Tryptase is a preformed neutral protease found in the secretory granules of human mast cells, and it is released together with other mediators when

the activation and consequent degranulation of the mast cells occurs¹. This enzyme has been identified in two of the three types of human mast cells described^{2,3}, but it is not present in other types of cells with the exception of the basophils, which contain it in minute quantities (300- to 700-fold less than in the lung and skin mast cells)⁴.

Activated mast cells secrete, besides tryptase, other preformed mediators, among them histamine, proteoglycans and another neutral protease (chymase), as well as *de novo* generated mediators such as prostaglandin D₂, leukotriene C₄ and platelet activation factor⁵. All these mediators are potentially useful as markers of mast cell activation. However, in opposition to the case of tryptase, these mediators either are produced by other cells besides the mast cell, so that their quantitation is not a specific marker of mast cell activation, or their quantitation in biological fluids is technically difficult.

Histamine, measured in plasma or in other body fluids, has until now been the best studied mast cell mediator. This compound has the following characteristics: it has a very short half-life, of only a few minutes, as it reaches its maximal concentration in the peripheral circulation only 10 - 15 minutes after the activation of the mast cells and returns to baseline values within 30 minutes; it is also present in considerable quantities in the basophils, so that it is not an exclusive marker of the mast cells, and an exceedingly careful handling of the samples is required in order to prevent haemolysis and basophil degranulation, which would lead to an artefactual increase of the circulating histamine levels.

In opposition to these particularities, tryptase evidences the following characteristics: although it is released in parallel to histamine from activated mast cells⁶, it is not usually detected in the circulation until 30 minutes later. This is probably due to its low diffusion rate through the extracellular matrix, as it complexes with proteoglycans. Its *in vivo* half-life is 2 hours, with peak levels in the circulation *ca.* 1 to 2 hours after its release, and it does not return to baseline values in the peripheral circulation until 4 to 6 hours later¹⁻⁷. Its concentration is not significantly affected in serum samples which have undergone several freeze-thaw cycles or after incubation for up to four days at room temperature and at 37°C⁸. Finally, the amount of tryptase derived from the basophils is negligible, so that careful handling of the blood samples after extraction is not required.