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Risk Factors for Allergy due to the Two-Spotted Spider Mite *(Tetranychus urticae)* among Table Grape Farm Workers

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Key Words
Occupational allergy · Work-related asthma · Spider mite · Grape farmers · Pesticides

Abstract

Background: Recent studies have reported an increased prevalence of respiratory symptoms among farm workers. The aim of this study was to identify risk factors associated with spider mite allergy among table grape farm workers. ***Methods:*** A cross-sectional study of 207 workers in table grape farms was conducted. Skin prick tests used extracts of 8 common aeroallergens, grape mould *(Botrytis cinerea)* and *Tetranychus urticae*. Specific IgE to *T. urticae* was quantified using ImmunoCAP (Phadia). Erythrocyte cholinesterase levels were determined using the Test-mate ChE Cholinesterase Test System (model 460). ***Results:*** The average duration of employment of workers was 10 years and 12% were pesticide crop sprayers. Work-related wheeze (26%), ocular-nasal (24%) and urticaria/skin symptoms (14%) were more prevalent in the orchards. The prevalence of sensitization (skin prick test) was highest to *T. urticae* (22%) followed by house dust mite (16%), with 25% being atopic. The prevalence of allergy to *T. urticae* (skin reactivity and work-related symptoms) was 9.5%, with respiratory allergy (6%) more common than skin allergy (3%). Work-related ocular-nasal (OR = 4.9) and skin (OR = 3.7) symptoms were more commonly reported by pesticide crop sprayers. Workers with *T. urticae*-allergic rhino-conjunctivitis and probable asthma were more likely to be atopic, spray pesticides and have low (<30 U/g hemoglobin) erythrocyte cholinesterase levels. ***Conclusions:*** This study demonstrated that spider mite, *T. urticae*, is an important outdoor allergen among table grape farm workers. The increased risk of spider mite allergy appears to be related to high pesticide exposure among crop sprayers.

Introduction

Asthma and other respiratory health problems among farm workers may be either due to excessive agrichemical use, biological factors (animal, vegetable or micro-organisms and their contaminants such as endotoxins) or in all likelihood a combination of both [1]. Various studies among workers exposed to pesticides in vineyards and orchard workers demonstrate an increased prevalence of respiratory symptoms including asthma associated with

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decrements in lung function [2–8]. More recent studies among fruit farm workers suggest that outdoor phytophagous mites appear to play a major role in the allergic manifestations observed among this group [9].

There have been few epidemiological studies that have investigated the prevalence and risk factors for allergic sensitisation to spider mite and related adverse health outcomes among working populations. A cross-sectional study of citrus farm workers in Korea reported a prevalence of sensitisation to spider mite *(Panonychus citri)* of 16.5% and 12.1% were diagnosed as having occupational asthma [10]. Another cross-sectional study of 725 apple farmers demonstrated that *Panonychus ulmi* and *Tetranychus urticae* were the most common apple leaf spider mites causing isolated allergic skin responses in 8.6% of this group [11]. Studies in Spain reported a 25% prevalence of sensitisation to *T. urticae* among greenhouse workers, with sensitisation being significantly associated with atopic status [12]. In this study, 19% of the sensitised workers had allergic rhinitis symptoms and 7% had asthma symptoms. More recently, epidemiological studies have demonstrated that spider mites are also important allergens causing or aggravating asthma and rhinitis symptoms among adults and children living close to citrus and pear farms in Korea [9].

Surveys on the ecology and distribution of outdoor mite populations in the Western Cape province of South Africa indicate that spider mites, *T. urticae* and *P. ulmi,* are important pests encountered in apple orchards [13]. The farming of wine and table grapes is one of the largest fruit industries in the Western Cape, employing about 50,000 workers in approximately 3,000 farms [14]. Vineyards in contrast to other fruit farms (citrus, apple and pear) have not been previously investigated for occupational allergy and asthma due to spider mites, although sensitisation to grapes has recently been studied [15]. Since these vine farms are located within the same geographical region as apple farms, the ecological distribution of spider mites in these vineyards was considered in all likelihood to be very similar to apple farms. Furthermore, the increased use of anti-mite pesticides (e.g. organophosphates and carbamates) using tractors with mist blowers in table grape farms as opposed to wine grape farms made this group of workers the preferred target group for further investigation [14].

The main objective of this study was to determine the spectrum of allergic sensitisation to various allergens, the prevalence of work-related allergy and asthma and the risk factors associated with spider mite allergy among workers on table grape farms.

Material and Methods

Study Design

A cross-sectional study was conducted on 207 workers employed in table grape farms in the Hex River valley in the Western Cape of South Africa. The workers for the study were randomly selected from 9 suitable table grape farms previously identified for a study on pesticide usage on these farms.

Questionnaire

The main study instruments included a questionnaire and skin prick testing (SPT). Each worker answered a standard abbreviated questionnaire based on the European Community Respiratory Health Survey questionnaire, slightly modified for local conditions [16]. This was administered by a trained interviewer in the language of the worker. Questions covered work history, job task, symptoms at work and medical history including medication use in the past year. Symptoms such as wheezing, ocular-nasal symptoms, as well as skin redness/itching/eczema experienced in the past year were considered to be work related if they were reported by the workers to be associated with working in the farm orchard or storeroom. Asthma was defined as a positive answer to the following question [17]: Has a doctor ever diagnosed you as having asthma?

Skin Prick Tests

SPT was performed on 190 of the 207 investigated workers, excluding pregnant workers and those with acute asthma symptoms. The commercial extracts (ALK) used were as follows: house dust mite *(Dermatophagoides pteronyssinus)*, mould mix *(Cladosporium herbarum, Alternaria alternata, Fusarium)*, bermuda grass *(Cynodon dactylon),* rye grass *(Lolium perenne)*, cat *(Felis domesticus)*, tree mix (false acacia, live oak, olive, white birch, ash) and cockroach *(Blatella germanica)*. Potential occupational allergens tested included grape mould *(Botrytis cinerea)* and an in-house-generated extract of the two-spotted spider mite, *T. urticae*. Histamine dihydrochloride was used as positive control and a diluent of glycerol/sodium chloride as negative control. Areas of erythema were traced on clear tape that was stored for later verification. A positive test was regarded as a wheal read 15 min after testing that had an allergen/histamine wheal ratio ≥ 0.5 [18]. The examiner was blinded to the exposure status of each worker. A subject was considered to be atopic if there was a positive skin reaction to one or more common inhalant allergens [19]. Occupational sensitisation to spider mite was defined as positive SPT to *T. urticae* with an allergen/histamine wheal ratio ≥ 0.5.

IgE Antibody Measurements

Specific IgE levels were quantified in serum obtained from 201 workers using the ImmunoCAP system (Phadia, Uppsala, Sweden) for house dust mite *(D. pteronyssinus)*, storage mite *(Lepidoglyphus destructor)* and the two-spotted spider mite *(T. urticae)*. According to the manufacturer's manual, a result was considered positive for any value greater than 0.35 kU/l.

Erythrocyte Cholinesterase Levels

Most organophosphate or carbamate pesticides inhibit blood enzymes erythrocyte acetylcholinesterase (AChE) and/or plasma cholinesterase [20]. In general, the degree of enzyme inhibition is proportional to the extent of pesticide exposure. In this study,

AChE levels were determined using a finger prick method, using the Test-mate ChE Cholinesterase Test System (model 460; EQM Research Inc.). Since serial measurements of AChE were not available for this group of workers, blood cholinesterase levels below 30 U/g hemoglobin (Hgb; 2 U/g Hgb lower than the mean levels for the high pesticide exposure jobs) were considered as being reduced for this study population.

Statistical Analysis

Statistical analysis was performed using Stata software (version 8; Stata Corp., College Station, Tex., USA) for univariate, bivariate and multivariate logistic regression analysis of the outcomes in relation to the predictors of interest. In the descriptive statistics, the means and standard deviations for each variable are presented since the exploratory data analysis revealed a normal distribution. The χ^2 test was used for categorical data and odds ratios (OR) were computed for the logistic regression models. Host factor variables entered into the models included age, gender, smoking status and atopy. Environmental risk factor variables explored in the regression models included employment duration and job type categorised into pesticide exposure categories: low = caretaker/creche minder/manager; medium = general farm labourers; high = tractor drivers/crop sprayers.

Ethical Considerations

The Research Ethics Committee of the University of Cape Town approved the study. Informed written consent was sought prior to any investigations being performed on any of the workers.

Results

Demographic Characteristics

The demographic characteristics of the study population are outlined in table 1. The proportion of males to females in this study population was very similar. The average duration of employment of workers on these grape farms was 10 years, most of them commonly employed in the same job during this period. Twelve percent of the workers were primarily involved in pesticide crop spraying using tractors. This group of workers also had the lowest mean AChE levels (32 ± 8 U/g Hgb; range: 22–51 U/g Hgb), when compared to the non-crop sprayers in the medium (34 ± 8 U/g Hgb; range: 5–59 U/g Hgb) and low (36 ± 4 U/g Hgb; range: 29–40 U/g Hgb) pesticide exposure groups. Only 1 worker in the low pesticide exposure group had 'depressed' (<30 U/g Hgb) AChE levels.

SPT and IgE Reactivity

The prevalence of sensitisation to common inhalants is shown in table 2. The prevalence of atopy was relatively low (25%), while skin reactivity to spider mite *T. urticae*

Table 1. Demographic characteristics of table grape farm workers (n = 207) in South Africa

Age, years	36 ± 11
Gender, F:M	1.1:1
Smoking, %	
Non-smokers	16
Ex-smokers	22
Current smokers	62
Employment status, % permanent	86
Employment duration in current farm, years	10 ± 8
Employment in current job, years	9 ± 8
Employment duration in other grape farms, years	5 ± 5
Pesticide crop sprayers, %	12
Cholinesterase (AChE) levels, U/g Hgb	34 ± 7
Low pesticide exposure (n = 5)	36 ± 4
Medium pesticide exposure (n = 145)	34 ± 8
High pesticide exposure (n = 22)	32 ± 8
Physician-diagnosed asthma	9
Asthma medication usage, %	7

Pesticide exposure categories: low = caretaker, creche minder, manager; medium = general workers; high = tractor drivers, crop sprayers.

Table 2. Prevalence of IgE reactivity to common inhalants and potential occupational allergens among table grape farm workers in South Africa

Allergen	Prevalence
SPT (n = 190)	
Common inhalants	
House dust mite *(D. pteronyssinus)*	16
Cockroach *(B. germanica)*	11
Rye grass *(L. perenne)*	11
Bermuda grass *(C. dactylon)*	5
Mould mix *(C. herbarum, A. alternata, Fusarium)*	3
Tree mix (false acacia, live oak, olive, white birch, ash)	3
Cat *(F. domesticus)*	2
Occupational allergens	
Spider mite *(T. urticae)*	22
Grape mould *(B. cinerea)*	2
ImmunoCAP (n = 201)	
House dust mite *(D. pteronyssinus)*	20
Spider mite *(T. urticae)*	16
Storage mite *(L. destructor)*	13
Atopy	25

Prevalence of sensitization is presented as percentage of positive results. Atopy = Sensitisation to common inhalants (SPT).

Table 3. Prevalence of mite-allergic outcomes among table grape farm workers in South Africa

Symptom/outcome	General symptoms	Work-related symptoms	Work-related mite-allergic outcomes				
			spider mite		house dust mite		storage mite
			SPT	ImmunoCAP	SPT	ImmunoCAP	ImmunoCAP
	(n = 207)	(n = 207)	(n = 190)	(n = 201)	(n = 190)	(n = 201)	(n = 201)
Sensitisation	–	–	42 (22)	32 (16)	31 (16)	40 (20)	27 (13)
Skin/urticaria	41 (20)	30 (14)	6 (3)	6 (3)	6 (3)	6 (3)	3 (1)
Ocular-nasal/rhino-conjunctivitis	52 (25)	49 (24)	12 (6)	10 (5)	13 (7)	14 (7)	8 (4)
Wheeze/probable asthma	63 (30)	54 (26)	11 (6)	10 (5)	10 (5)	15 (7)	12 (6)
Doctor-diagnosed asthma	–	19 (9)	4 (2)	3 (1)	3 (2)	6 (3)	5 (2)

Figures in parentheses are percentages.

was more prevalent (22%) than to house dust mite (16%), with 6% demonstrating monosensitivity to spider mite. Only few (2%) workers demonstrated sensitisation to grape mould *(B. cinerea)* on SPT. Quantification of mite-specific IgE by ImmunoCAP demonstrated the highest prevalence of elevated IgE levels for house dust mite (20%), followed by spider mite (16%) and storage mite (13%).

While only 36% of the 42 workers with positive skin prick reactivity to spider mite had elevated specific IgE levels on ImmunoCAP, a statistically significant but low correlation was demonstrated between these 2 indices of allergic sensitisation (Spearman $R = 0.32$, $p < 0.001$). After stratifying the data, a much stronger level of agreement was obtained between spider mite ImmunoCAP and SPT with an allergen/histamine wheal ratio between 1 and 2 ($\kappa = 0.30$, $p < 0.001$), than for an allergen/histamine wheal ratio between 0.5 and 1 ($\kappa = 0.14$, $p < 0.05$; data not shown), suggesting that the SPT with a lower cut-off for the allergen/histamine wheal ratio had a higher degree of sensitivity but reduced specificity compared to the specific serum IgE levels on ImmunoCAP. Furthermore, while ImmunoCAP results of house dust mite strongly correlated with storage mite (Spearman $R = 0.71$, $p < 0.001$), they only modestly correlated with spider mite (Spearman $R = 0.49$, $p < 0.001$). The correlation of IgE reactivity between spider mite and storage mite was also modest (Spearman $R = 0.61$, $p < 0.001$).

Work-Related Symptoms and Mite-Allergic Outcomes

The prevalence of work-related wheezing (26%) and ocular-nasal symptoms (24%) was more common than urticaria/skin symptoms (14%; table 3). Nine percent of workers reported physician-diagnosed asthma. When compared to general symptoms, the prevalence of work-related symptoms was not significantly different for wheezing (26 and 30%), ocular-nasal (24 and 25%) and skin symptoms (14 and 20%). However, the prevalence of work-related symptoms was higher when working in the orchards compared to the stores (χ^2 test, $p < 0.001$).

The overall prevalence of spider mite allergy (work-related allergic respiratory symptoms and sensitisation to *T. urticae* on SPT) was 9.5%, with rhino-conjunctivitis and probable asthma (5–6%) more common than skin allergy (3%; table 3). The prevalence of work-related spider mite-associated probable asthma (5–6%) was very similar to house dust mite-associated asthma (5–7%). The prevalence of doctor-diagnosed asthma due to spider mite (1–2%) and house dust mite (2–3%) was also similar but lower.

Risk Factors for Work-Related Symptoms, Sensitisation and Spider Mite Allergy

Work-related skin symptoms including urticaria (OR = 3.7) as well as ocular-nasal symptoms (OR = 4.9) were more likely reported by pesticide sprayers (table 4). This is consistent with the 2- to 3-fold increased likelihood of those workers with low (<30 U/g Hgb) cholinesterase levels reporting work-related skin symptoms.

Analysis of risk factors for spider mite-related allergy (allergic symptoms and SPT reactivity; table 5) indicated that workers with *T. urticae*-specific rhino-conjunctivitis and probable asthma were at least 4 times more likely to be atopic or pesticide crop sprayers. Similar results were obtained when using the ImmunoCAP results as the outcome variable. Furthermore, workers with *T. urticae*-specific rhino-conjunctivitis were more likely (OR = 4.5) to have low (<30 U/g Hgb) cholinesterase levels.

Discussion

This is the first study to demonstrate sensitisation to spider mites among table grape farmers. A high prevalence of sensitisation to *T. urticae* (22%) on SPT was found, with 6% of workers demonstrating isolated positive skin responses to spider mite. This prevalence is lower than in studies among citrus farmers (16.5%) but comparable to apple farmers (5%) in Korea [10, 11]. Considering the existence of other potential sources of common allergens on farms, the higher prevalence of sensitisation to spider mite among table grape farm workers compared to allergens such as the indoor domestic mites (house dust mite and storage mite) was an unexpected finding. This could be due to the nature of work involved, in that outdoor farming promotes the development of spider mite populations due to favourable temperature and humidity conditions. This could explain the increased prevalence of work-related respiratory symptoms reported by workers when working in the orchard compared to working indoors in the stores as has been found in other studies [5]. Furthermore, in recent years the spider mite *T. urticae* has proliferated extraordinarily worldwide, including the South African farm environment, probably due to its resistance to common pesticides [13, 21, 22].

Physician-diagnosed asthma was reported by 9% of workers, which is similar to the current estimated prevalence of 8.1% for clinical adult asthma in South Africa [23]. However, table grape farm workers in South Africa reported a higher prevalence (26%) of work-related wheezing in the past year compared to the 19% prevalence recently reported by pesticide applicators in the USA [2]. Wheezing and ocular-nasal symptoms were more frequently reported in both spider mite- and house dust mite-allergic workers. These findings demonstrate that spider mite-related allergic symptoms appear to be as common as house dust mite allergies, especially for outdoor work-related activities. It is also evident that the commonly held view that the grape mould *(B. cinerea)* is an important allergen in grape handling is not borne out by the findings in this study [24]. The contribution of grape allergen or vine pollen sensitisation to allergic symptoms is another factor to consider in explaining the allergic symptoms reported [15, 25]. This, however, was not assessed since no well-validated SPT extracts were available at the time of the study.

In this study, 57% (24/42) of the workers with a positive SPT to *T. urticae* were asymptomatic. The clinical significance of sensitisation in asymptomatic workers is unknown, but this may identify a group at risk of presenting with allergic symptoms in the future. Since the current study was conducted in winter, when the environmental exposure to spider mite is low, this may also have influenced the low prevalence of respiratory symptoms reported by workers, as has been demonstrated in other studies [26]. As with most cross-sectional studies, this may also be indicative of a healthy worker effect in operation, in that symptomatic workers may have left the job and sought alternative employment.

Table 4. Important risk factors for work-related symptoms among table grape farm workers in South Africa

Outcome and predictor	Prevalence OR	Confidence interval	p value
Urticaria/skin			
Pesticide crop sprayer[1]	3.7	0.9–13.5	0.051
Low cholinesterase (AChE)	3.2	1.3–7.6	0.009
Low cholinesterase (AChE)[1]	2.6	0.9–7.2	0.059
Ocular-nasal			
Male	1.9	1.0–3.7	0.048
Pesticide crop sprayer[1]	4.9	1.6–14.7	0.004
Wheeze			
Age	1.0	1.0–1.1	0.031
Current smoker	3.3	0.9–11.7	0.065

Low cholinesterase (AChE) was defined as AChE <30 U/g Hgb.

[1] Adjusted for age, gender, smoking and atopy (sensitisation to common inhalants on SPT).

Table 5. Important risk factors associated with work-related spider mite respiratory allergy among table grape farm workers in South Africa

Outcome and predictor	Prevalence OR	Confidence interval	p value
Rhino-conjunctivitis			
Atopy	4.7	1.4–15.5	0.012
Low cholinesterase (AChE)[1]	4.5	1.1–18.7	0.041
Pesticide crop sprayer[1]	4.4	0.9–20.1	0.059
Probable asthma			
Atopy	9.3	2.3–36.6	0.001
Pesticide crop sprayer[1]	4.5	0.9–22.1	0.063

Low cholinesterase (AChE) was defined as AChE <30 U/g Hgb.

[1] Adjusted for atopy (sensitisation to common inhalants on SPT).

Symptomatic workers were also frequently sensitised to house dust mite or storage mites, thereby raising the question of cross-reactivity. A high degree of correlation (Spearman R = 0.71) was demonstrated between elevated IgE levels to house dust mite and storage mite, however, a much lower degree of correlation was found between house dust mite and spider mite (Spearman R = 0.49). This can be explained by the high degree of cross-reactivity demonstrated between the domestic house dust mite and closely related storage mite, belonging to the same phylogenetic sub-order (Astigmata) [9]. On the other hand, inhibition studies have demonstrated limited cross-reactivity between house dust mite and spider mite due to few shared allergens since they belong to the sub-order Prostigmata [27, 28]. Up to 10 different IgE binding components have been identified by immunoblotting and subsequent sequencing, suggesting that the major allergens are different from domestic mites [29]. Interestingly, in addition to the investigated mite species, various predatory mites (sub-order Mesostigmata) are also commonly found in South African fruit farms, containing species-specific allergens not shared with spider or domestic mites that are also capable of inducing symptoms among exposed workers [13, 30]. Sensitisation to these predator mites among fruit farmers, however, has not been previously investigated in detail as has been the case for greenhouse workers. Future studies will need to compare the IgE binding profiles of spider and predator mite-exposed table grape farmers with those identified among symptomatic apple and pear farmers and greenhouse workers. This will enable the identification of common and specific major allergens that will contribute towards improving the diagnostic modalities for investigating work-related asthma among fruit farmers.

This current study in the Western Cape province of South Africa demonstrated a high prevalence of general wheeze (30%) as well as work-related wheeze (26%) among table grape farm workers compared to previous studies in this province [3, 4]. This is higher than the 18% prevalence of wheeze reported among grape farmers in Greece, despite a much higher prevalence of atopy (64%) observed in this latter population [31]. Interestingly, while the prevalence of atopy was much lower (25%) and not significantly associated with general work-related symptoms in the South African study, it was nevertheless a consistent and significant host risk factor for *T. urticae*-allergic symptoms. This finding confirms previous studies demonstrating atopy as an important predisposing factor for *T. urticae* hypersensitivity [12].

Furthermore, this study also demonstrated that table grape farm workers with work-related upper respiratory symptoms were more likely to be pesticide crop sprayers (OR = 4.9, p = 0.004). The borderline significant adjusted OR for work-related skin symptoms and pesticide crop spraying (OR = 3.7, p = 0.051) and low AChE levels (<30 U/g Hgb; OR = 2.6, p = 0.059) are also suggestive of a possible association. However, the study by Chatzi et al. [32] among grape farmers in Greece demonstrated a high prevalence (18%) of work-related skin symptoms, but was unable to demonstrate an association with pesticide use. The associations for respiratory outcomes were consistent even with spider mite-allergic rhino-conjunctivitis and probable asthma as the outcomes of interest. The current study highlights the potential influence of pesticides such as organophosphates, commonly used in table grape farms, on the development of allergic sensitisation [33]. It has increasingly been demonstrated over the last decade that some environmental chemicals do not only have direct irritant effects but also act as endocrine disruptors, directly affecting the immune system [34]. Recent murine studies on the effect of endocrine disruptors on T cells have clearly demonstrated a Th2-induced polarisation resulting in enhanced allergic sensitisation to allergens [35, 36].

These findings suggest that the two-spotted spider mite, *T. urticae*, could be considered an important mite allergen responsible for work-related asthma symptoms among table grape farm workers in South Africa and possibly in other grape and fruit farms where large populations of spider mites are prevalent. The dose-response relationships, however, need further refinement through further exploration of the major route/s of sensitisation and characterisation of environmental aeroallergen concentrations of spider mite exposures in various jobs performed by workers. The contribution of storage and possibly house dust mite sensitisation to work-related asthma symptoms needs to be investigated further for probable cross-reactivity to spider mite allergens in this setting. The background prevalence of sensitisation to spider mite in the general population and sensitisation to vine pollen among grape farmers will also shed more light on the determinants of work-related symptoms among this group of workers. This should be accompanied by determination of lung function airway parameters to corroborate the asthma symptoms reported in this study. Further studies need to examine more closely the relationship between sensitisation to spider mite and excessive pesticide use as a possible mechanism for causing work-related asthma.

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