Annals of Parasitology 2017, 63(3), 183–188
doi: 10.17420/ap6303.104

Original papers

Sensitization to the storage mites *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* (Acari, Sarcoptiformes, Astigmatina) in a suburban population in Southern Poland

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ABSTRACT. Mite infestation of stored products is a serious threat to food safety and public health. These stored product mites are not only serious pests of stored food but also cause allergies in humans. Thirty serum samples from patients living in suburban areas of Upper Silesia (South Poland) were tested for sensitization to two species of storage mites: *Lepidoglyphus destructor* [LD] and *Tyrophagus putrescentiae* [TP]. Patient antibodies against particular antigens were identified using anti-human anti-IgE monoclonal antibodies. Fifteen protein fractions from LD gave positive reactions with IgE antibodies and 18 from TP. Seven of the 30 samples showed positive reactions to a protein fraction measuring about 29 kDa from LD and six reacted with a fraction measuring about 25 kDa from TP. These findings may imply the existence of many protein fractions with allergenic properties besides the characterized allergens in the two tested species.

Key words: storage pests, mites allergens, occupational allergy, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*

Introduction

Stored product mites are found in house dust samples but are also serious pests of stored food, and are found on a variety of products including grain, flour, hay and straw. These storage mites are widely known to produce allergens, and may be a cause of asthma and other allergic reactions, including anaphylaxis, after ingestion of contaminated foodstuffs [1,2]. *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* are not only two of the most widespread species of storage mite, but they also represent the most important source of allergens in the rural environment in Europe. They occur mostly in humid housing conditions, and although allergy to storage mites is of major importance in rural areas, especially among farm workers, millers and bakers, many cases of sensitization have been reported in urban populations without occupational exposure [1–5].

The most significant allergens of the two species have been extensively studied, with Lep d 2 being the most active allergen of *L. destructor* [6,7] and Tyr p 2 of *T. putrescentiae*. The most significant source of mite allergens are their faeces [8–10], where the most important classes of mite allergens, their digestive enzymes, are found [11,12].

The aim of this study was to determine the recognition frequency of potential allergenic protein fractions from *L. destructor* and *T. putrescentiae*, two of the most common and significant sources of allergens among storage mites in Europe, among individuals living in suburban areas.

Materials and Methods

The study was approved by the local governing human research protection committee (Medical University of Silesia in Katowice; protocol number KNW/022/KB/86/1/15).

Prepared extracts of *L. destructor* and *T. putrescentiae* (Allergopharma, Germany) were used. Serum samples were collected from 30 Health Care Centre patients from the suburban population

Table 1. Protein fractions revealed in the examined extracts of *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* as antigens reacting with sera of the examined subjects from suburban population of Southern Poland

	Species of storage mite	
	Lepidoglyphus destructor	*Tyrophagus putrescentiae*
Protein fraction [kDa]	25	20
	26	25
	27	26
	29	28
	30	29
	31	30
	37	31
	44	32
	62	36
	66	46
	67	50
	68	56
	69	62
	70	66
	98	68
		70
		71
		98

of Upper Silesia (South Poland). The samples were obtained as waste material after routine laboratory diagnostic testing from a range of sources, *viz.* a dermatology clinic (n=3), laryngology clinic (n=4) and primary health care clinic (n=23). All sera were stored in aliquots at –80°C until use.

The extracts of *L. destructor* and *T. putrescentiae* were separated by SDS-PAGE and electro-blotted to nitrocellulose.

The SDS-PAGE was performed using a mini-Protean II system (BioRad, USA) according to Laemmli [13], with some modifications. The electrophoresis was performed for 90 minutes at 100 V using 12% separating gels. The gels were then electrotransferred using a Mini-Transblot Cell (BioRad, USA) according to Towbin et al. [14] with modifications. Electrotransfer was performed at 150 mA for one hour. After being blotted, the nitrocellulose membranes were blocked overnight with casein milk and then incubated at 4°C with 150 µl of human sera diluted 1:100 in Tris Buffered Saline (TBST). The samples were washed in TBST three times for 15 minutes, and incubated for two hours at room temperature with anti-human IgE (Sigma-Aldrich, Germany), diluted 1:1000 in TBST.

The samples were washed three times in TBST for 15 minutes and then in AP Buffer for five

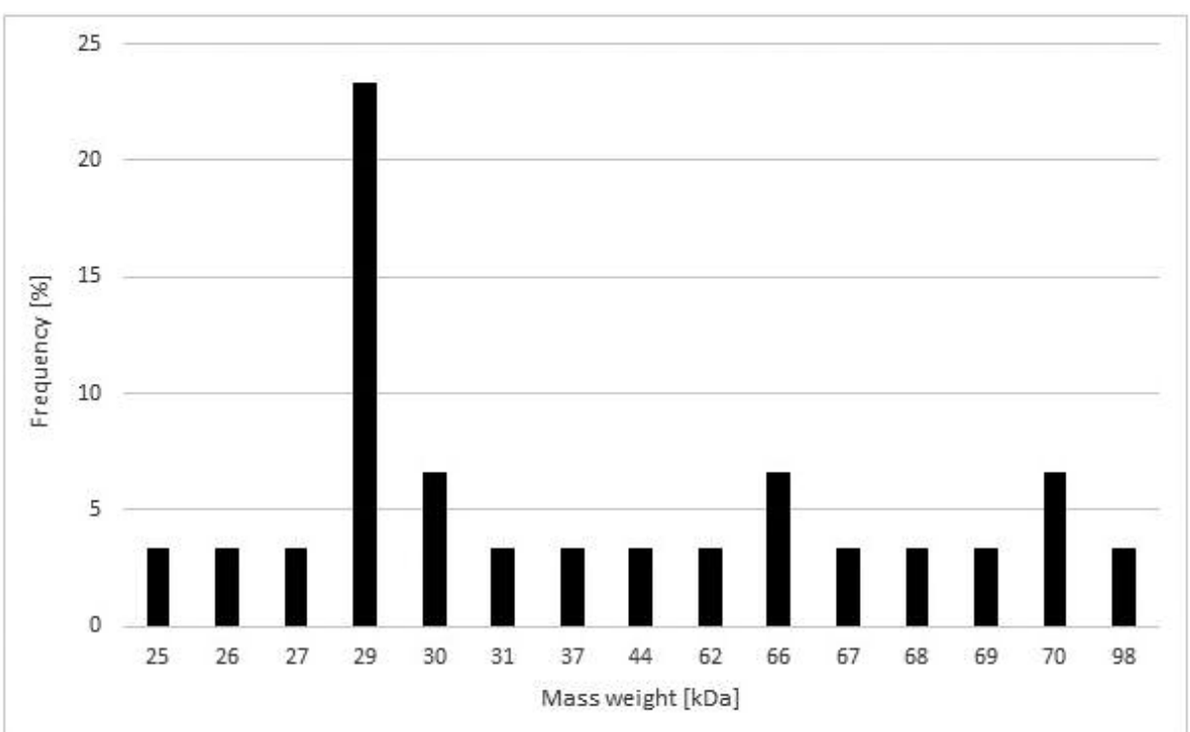

Fig. 1. Percent of sera giving positive reactions with antigens in the examined extracts of *Lepidoglyphus destructor*

minutes. The membranes were incubated in BCIP/NBT Liquid Substrate System (Sigma-Aldrich, Germany) for 20 minutes. The air-dried probe membranes were then analysed using the Omega 10 Analyzer (UltraLUM, USA) and the results developed in Total Lab software (Total Lab, USA). Comparison of the two species of storage mites was performed using the Yates corrected Chi-square test.

Results

Sixteen of the 30 tested sera (53.3%) demonstrated positive reactions to the protein fractions of *L. destructor* or *T. putrescentiae*. Fourteen sera showed positive reactions to the *L. destructor* fractions and 15 to the *T. putrescentiae* fractions.

Eighteen protein fractions from *T. putrescentiae*, and 15 from *L. destructor* were observed in the examined sera (Table 1).

For the *L. destructor* extracts, 23.3% of patients demonstrated a reaction with a 29 kDa fraction. It should be stressed that this percentage was significantly higher than the 6.6% who reacted to the 30, 66 or 70 kDa fractions (Fig. 1) (Yates corrected $\chi^2 = 8.82$; $p = 0.003$).

In case of *T. putrescentiae*, the most common reactions were with the 25 kDa (20%) and 30 kDa (13.3%) protein fractions: this difference was statistically insignificant (Yates corrected $\chi^2 = 1.31$; $p = 0.25$). Only 6.6% of the patients reacted to either the 26, 28, 29, 66, 70 or 71 kDa fraction (Fig. 2); however, while the difference between the proportions of subjects who responded to the 30 kDa fraction and to the seven other fractions was statistically insignificant (Yates corrected $\chi^2 = 1.39$; $p = 0.24$), the difference between the proportion responding to the 25 kDa fraction and the seven other fractions was statistically significant (Yates corrected $\chi^2 = 6.17$; $p = 0.013$).

Discussion

A considerable amount is known about house dust mite allergy, and the major allergens from this group of organisms are well characterized. Despite this, much remains unknown about allergies to storage mites, their allergens and their allergenic cross-reactivity with other mites [5]. Sensitization to storage mites in an urban environment has been reported in many European countries, including Spain, Denmark, Germany, Croatia and Poland [3,5,15–17].

Cichecka et al. [15] reported significantly greater sensitization to *L. destructor* and *T. putrescentiae* in

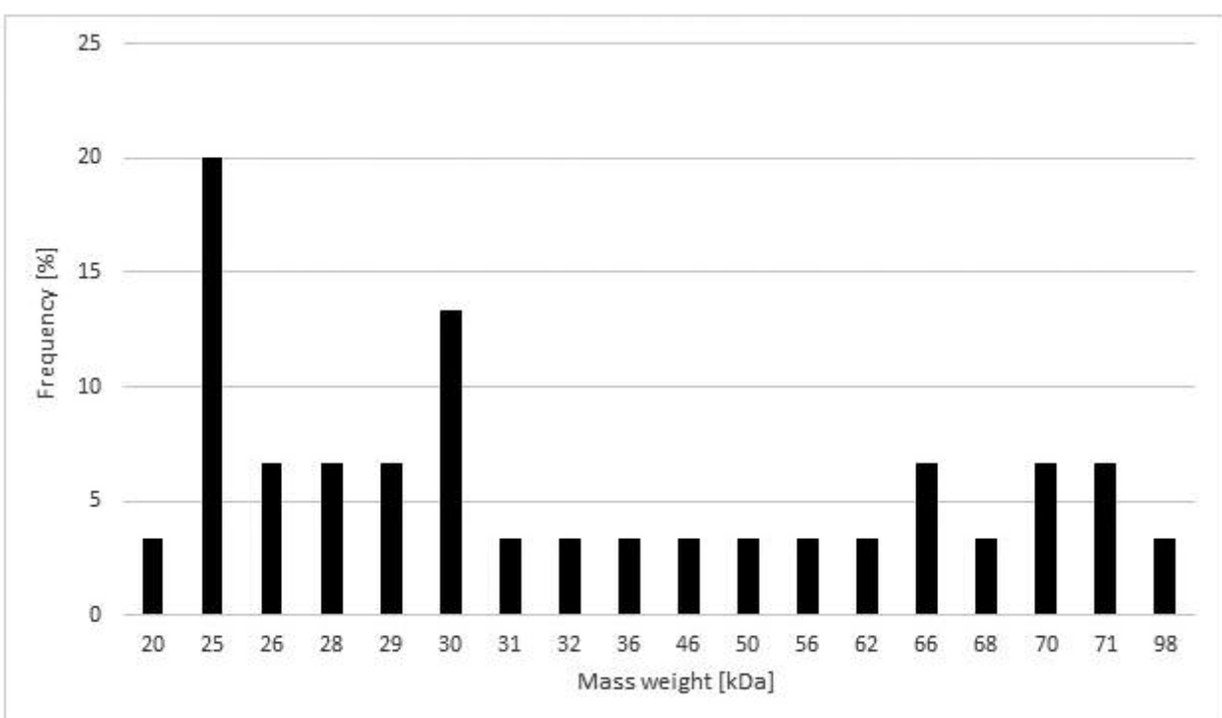

Fig. 2. Percent of sera giving positive reactions with antigens in the examined extracts of *Tyrophagus putrescentiae*

a sub-agricultural population, as well as in an urban group. In a study of dairy farmers, Marx et al. [18] found the most frequently observed antigens in a test panel to be IgE antibodies to house dust and storage mites, particularly *L. destructor*. In a study of 196 patients from urban areas who were not occupationally exposed to storage mites, Luczynska et al. [5] identified a 24% prevalence of the specific IgE antibody to *Dermatophagoides pteronyssinus*, a very common house dust mite, but also a 14% prevalence of RAST positivity to at least one of three common storage mites: *Acarus siro*, *L. destructor* or *Tyrophagus longior*. All patients who demonstrated a positive RAST score for storage mites also had a specific IgE to *D. pteronyssinus*. Their findings suggest that at least some of the response to storage mites was a consequence of cross-reactivity with the more frequent *D. pteronyssinus*.

Of a group of 82 mite-sensitive patients tested by Liao et al. [19], 81 were sensitive to *D. pteronyssinus* and 34 sensitive to *T. putrescentiae*. Among the patients sensitive to *T. putrescentiae*, 97% were also sensitive to *D. pteronyssinus*. Their research suggests that *T. putrescentiae* hypersensitivity could be as a result of cross-reactivity, rather than dual-sensitization of the two species.

Our findings reveal a 53.3% prevalence of IgE antibodies to *L. destructor* and *T. putrescentiae* within all the tested sera. Of all the *L. destructor* protein fractions, the largest percentage of sera reacted to the 29 kDa fraction, while in the *T. putrescentiae* extracts, the most commonly-observed reactions were with the 25 kDa and 30 kDa protein fractions. The results are in line with those of Szilman et al. [20], where 40% of the tested sera reacted specifically with protein fractions of extracts from *T. putrescentiae* excrement, and a protein of about 25 kDa was also identified.

While our present findings indicate 14 distinct antigens in the extract from *L. destructor*, a similar study of urban and rural populations from the province of Upper Silesia (south-west Poland) by Solarz et al. found only 12 [21]. Their results showed that sensitization to *L. destructor* was present in the urban population, and the proportions of the population sensitized to it were similar to those seen for the house dust mites *Dermatophagoides farinae* and *D. pteronyssinus* [21], which are much more common in this environment.

Our results indicate the presence of a 25 kDa protein fraction in *L. destructor*, which may be Lep d 7, judging by its mass. Eriksson et al. [22] reported that Lep d 7 was recognized by 62% of sera from *Lepidoglyphus*-sensitized patients and its calculated molecular mass is 22 kDa. The allergen showed homology with Der p 7 and Der f 7.

In the present study, a 44 kDa protein fraction was also found, which may correspond to Lep d 10, a 40 kDa allergen first isolated by Saarne et al. [23] and named based on its homology to the group 10 dust mite allergens.

Our analysis also identified a 98 kDa antigen, which may correspond to a high molecular weight allergen complex (79 and 93 kDa) described in *L. destructor* by Olsson et al. [24] and van Hage-Hamsten et al. [25].

Similarly, a 37 kDa fragment was found to be present in *L. destructor*; it is possible that this fragment could correspond to a 39 kDa protein detected elsewhere [26,27]. Van Hage-Hamsten et al. [27] found that 46.5% of serum samples from farmers who were RAST positive to *L. destructor* were also positive for this 39 kDa protein.

Some of the patients in the present study reacted to a 26 kDa protein fraction in the *T. putrescentiae* extracts. This fraction may be Tyr p 8, a 26 kDa protein which has been found to exhibit 83% sequence homology to Der p 8 and demonstrate 17.9% IgE-binding reactivity [28].

Another antigen identified in *T. putrescentiae* was about 32 kDa, which may correspond to Tyr p 10. This fragment has been found to have a mass of 32.95 kDa, and whose recombinant allergen demonstrates 12.5% IgE-binding reactivity [29].

Finally, a protein fraction of about 50 kDa was also identified in the present study. This protein closely resembles Tyr p 33, with a molecular mass of 50.4 kDa [30].

The obtained results may imply the existence of many protein fractions with allergenic properties besides hitherto characterized allergens in both tested species.

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Received 26 April 2017
Accepted 16 June 2017