



Sensitization to *Sitophilus granarius* in selected suburban population of South Poland



Justyna Jakubas-Zawalska^a, Marek Asman^{a,*}, Małgorzata Kłysz^b, Krzysztof Solarz^a

^a Medical University of Silesia in Katowice, Department of Parasitology, Jedności 8, 41-218 Sosnowiec, Poland

^b Pedagogical University of Cracow, Institute of Biology, Department of Ecology and Environmental Protection, Podbrzezie 3, 31-054 Kraków, Poland

ARTICLE INFO

Article history:

Received 26 January 2016

Received in revised form

19 May 2016

Accepted 19 May 2016

Keywords:

Insect allergens

Occupational allergy

Storage insects

Granary weevil

Sitophilus granarius

ABSTRACT

Storage insects may cause occupational allergies in humans. The identification of the prevalence of IgE reactions were measured from protein fractions prepared from multiple life stage of granary weevil *Sitophilus granarius* [SG] is reported. Sera of 30 patients from a suburban population of Upper Silesia (South Poland) were tested for the presence of IgE antibodies to antigens from larvae, pupae and adults of both sexes of the beetle. To identify protein fractions containing potential allergens, proteins collected from four life stages of granary weevil were fractionated by SDS-PAGE and probed with anti-human, anti-IgE monoclonal antibodies. The proteins were fractionated by SDS PAGE and identified by Western blot. The patients' antibodies against particular antigens were identified using anti-human anti-IgE monoclonal antibody. The conducted immunological analysis showed the existence of many protein fractions for each life stage of SG which give positive reactions with IgE antibodies. The largest number of allergenic potential fractions was shown in pupae (60 protein fractions) while the smallest amount was shown in larvae (44 protein fractions). Summarizing, the obtained results suggest the existence of many protein fractions with an allergenic potential multiple life stages of SG. This indicates that all developmental stages of SG may be a serious source of antigens and potential risk factors for the exposed persons.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Storage products and stores are very often infested by various pest arthropods that contaminate the environment with potent allergens and pathogens (Arlian, 2002; Campbell et al., 2004). Proteins from bodies, faeces, eggs, and secretions from some storage insects can cause allergic reactions in humans. Exposure to insect proteins can sensitize and induce IgE-mediated reactions. Allergies to stored product insects are among the most frequent allergies associated with arthropods (Arlian, 2002). The continuous exposure to insects in workplaces can lead to inhalant allergies which can manifest with asthma, but also atopic dermatitis, rhinitis, conjunctivitis and may be a cause of such occupational diseases as eczema or itching in bakers, millers, warehouse workers, farmers and other persons connected with agriculture (Alvarez et al., 1996; Revsbech and Dueholm, 1990).

Sitophilus granarius (L.) (Coleoptera: Curculionidae) is a very common pest of many types of grains and cereal products (Chambers and Collins, 2000; Cook and Armitage, 2002; Giacinto et al., 2008; Stejskal and Hubert, 2008; Woodbury, 2008). In addition to its great importance in food losses, it also has a significance in the medical and allergic aspects. This beetle is present in Europe, Africa, Asia, South and North America and Australia, and is most probably a cosmopolitan species. The life cycle includes an egg, larva, pupa and adults. Each stage can infest foods and may be a potential source of allergens. Though the granary weevil is one of the most common pests, knowledge about its allergens is still very poor. The aim of this research was to identify IgE-binding components in extracts of larvae, pupae, females and males of *S. granarius* to evaluate allergenic reactivity and differences between particular life stages.

2. Material and methods

S. granarius was bred in the laboratory conditions. The larvae, pupae, adults (males and females) of the insect were used to

* Corresponding author.

E-mail addresses: masman@sum.edu.pl (M. Asman), klysgosia@poczta.onet.pl (M. Kłysz).

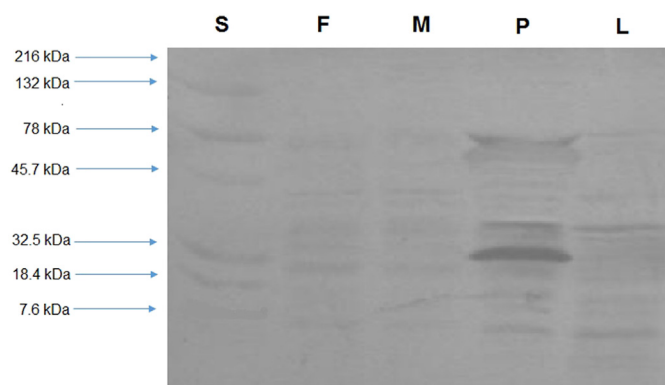


Fig. 1. The IgE response of the exemplary patient against protein fractions of *Sitophilus granarius*. Explanations: S – molecular weight standard (BioRad, USA); F – protein fractions of females; M – protein fraction of males; P – protein fractions of pupae; L – protein fractions of larvae.

prepare the whole body extracts. These four life stages of the weevil were tested. Five individuals of each life stage were pooled together and then used to prepare extracts.

Sera of 30 patients from the suburban population of Upper Silesia (South Poland) were examined. Human sera were collected in the Health Care Center as a waste material after routine laboratory diagnostic control. There was a protocol approved by the

local governing human research protection committee (protocol number KNW/022/KB/86/1/15). Samples included donors from health facilities ($n = 30$); including dermatology clinic ($n = 3$), laryngology clinic ($n = 4$) and primary health care ($n = 23$). All sera were stored in aliquots at -80°C .

Insect bodies were homogenized in Sample Buffer (SB) and denatured at 100°C for 5 min. The concentrations of proteins were measured by spectrophotometry on the nanospectrophotometer PEARL (Implen, Germany). The SDS-PAGE was performed as described by Laemmli (1970) with some modifications using a mini-Protein II system (BioRad, USA). The electrophoresis was performed at 100 V for 90 min using 12% separating gels. Next, gels were electrotransferred by a modified method of Towbin et al. (1979) using a Mini-Transblot Cell (BioRad, USA). Electrotransfer was carried out at 150 mA for one hour. Blotted nitrocellulose membranes were blocked overnight with casein milk and incubated at 4°C with human 150 μl of sera diluted 1:100 in Tris Buffered Saline (TBST). Next, samples were washed in TBST (3 times for 15 min) and incubated for 2 h at ambient temperature with anti-human IgE (Sigma-Aldrich, Germany), diluted 1:1000 in TBST. Then samples were washed in TBST (3 times for 15 min) and in AP Buffer for 5 min. The membranes were incubated for 20 min in BCIP/NBT Liquid Substrate System (Sigma-Aldrich, Germany). Air dried probe membranes were analysed using the Omega 10 Analyzer (UltraLUM, USA). The results were developed in the Total Lab computer program (Total Lab, USA) (Fig. 1).

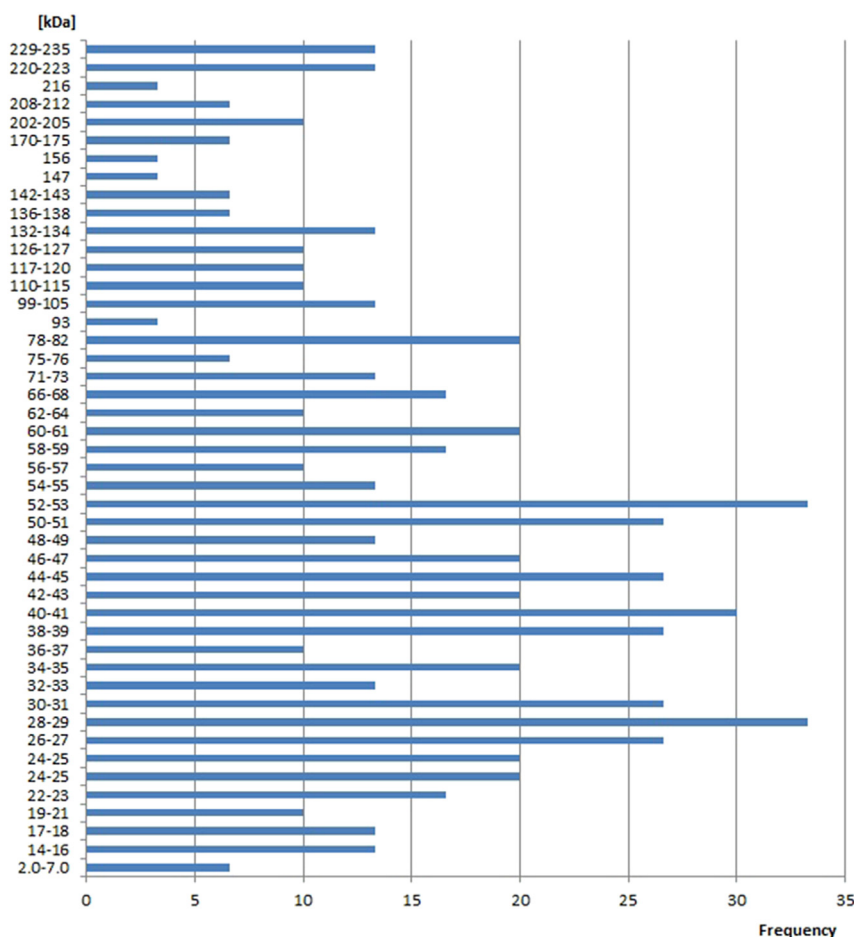


Fig. 2. Percent of sera giving positive reactions with antigens in the examined extracts of females *Sitophilus granarius*.

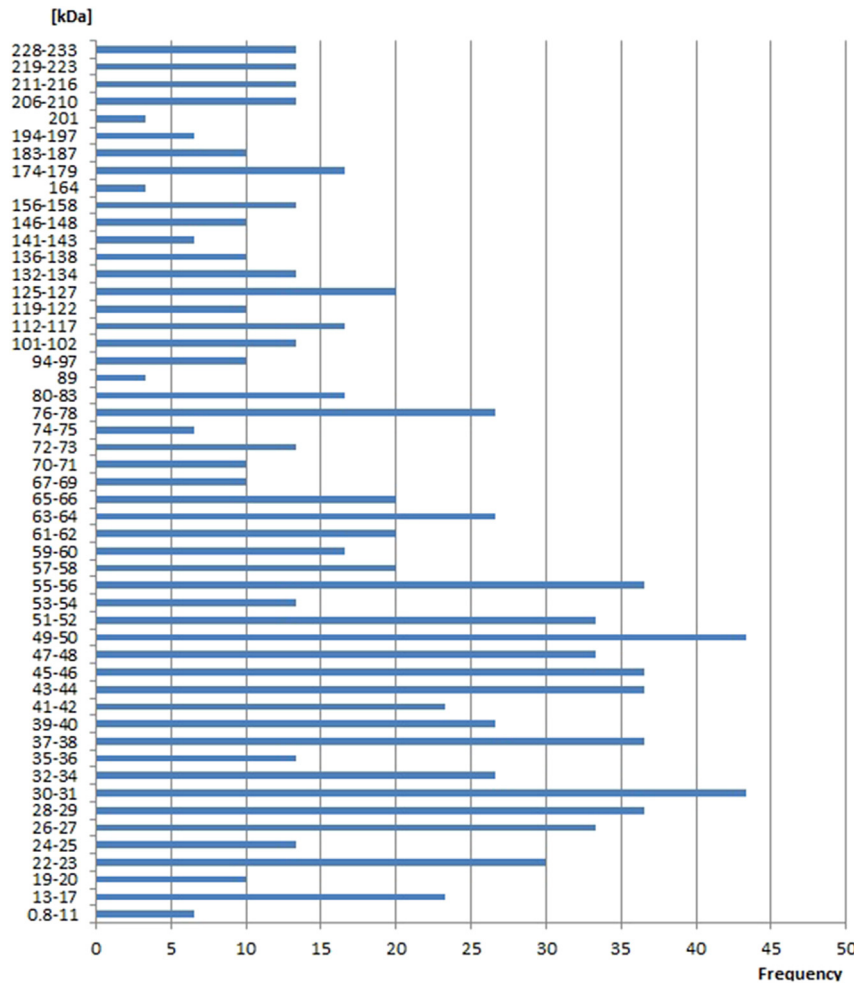


Fig. 3. Percent of sera giving positive reactions with antigens in the examined extracts of males *Sitophilus granarius*.

2.1. Statistical analysis

The statistical analysis was performed using CSS – Statistica for Windows version 12. Significance was declared at a P value of less than 0.05. Results were analysed using the χ^2 test.

3. Results

Generally, positive reactions to protein fractions of *S. granarius* were shown in sera collected from all 30 patients. However, western blot analysis revealed differences in reactivity of the examined sera with the particular fractionated life stages (Figs. 2–5).

The largest number of protein fractions in the examined sera was observed in pupae, whereas the smallest number was detected in larvae (Figs. 4 and 5). Among the four life stages, the largest percentage of the patients reacted to two fractions from pupae located at 27–28 kDa and 28–29 kDa (70% each). No other fraction from any other life stage exceeds this frequency. In case of pupae the largest amount of sera reacted with fractions located at 14–18 (46.6%), 25–26 (46.6%), 27–28 (70%), 29–30 (70%), 51–52 kDa (46.6%) (Fig. 4).

The most sera reacted with protein fractions located at 28–29, 40–41, 52–53 kDa in females and at 28–29, 30–31, 37–38, 43–44, 45–46, 49–50, 55–56 kDa in males of *S. granarius* (Figs. 2 and 3). Moreover, the protein fractions of larvae located at 26–27, 28–29, 30–31, 50–51 kDa showed the most frequent positive reactions (Fig. 5).

The smallest percentage of sera reacted with fractions located at 93, 147, 156 and 216 kDa in females, 89, 164 and 201 kDa in males, 240 and 258 kDa in pupae and 180, 202 and 233 kDa in larvae (Figs. 2–5).

Taking into account all tested life stages, examined sera reacted most frequently with two protein fractions which are about 30 kDa and about 50 kDa. The most frequent reactions with protein fractions at 50 kDa occurred in larvae (63.3%) followed by pupae (46.6%), males (43.3%) and females (33.3%). The most frequent reactions with protein fractions at 30 kDa occurred in pupae (70%) and larvae (63.3%) followed by males (36.6%) and females (33.3%).

Comparison of protein fractions located at about 50 kDa shows that sensitization to larvae extracts was statistically more frequent compared to pupal extracts (Yates corrected χ^2 test, $\chi^2 = 4.55$; $P = 0.033$), females (Yates corrected χ^2 test, $\chi^2 = 16.85$; $P \leq 0.00001$) and males (Yates corrected χ^2 test, $\chi^2 = 7.25$; $P = 0.0071$). However, frequencies of positive reactions for the 50 kDa protein fractions between pupae and females, pupae and males and adults of both sexes were not significantly different (Yates corrected χ^2 test, $\chi^2 = 3.52$, 0.18 and 1.72, respectively; $P > 0.05$). Comparison of protein fractions located at about 30 kDa shows that sensitization to pupae was statistically significant more frequent than to females (Yates corrected χ^2 test, $\chi^2 = 25.94$; $P \leq 0.00001$) and males (Yates corrected χ^2 test, $\chi^2 = 20.58$; $P \leq 0.0001$). Frequencies of positive reactions for the 30 kDa protein fractions were different between larvae and females and larvae and males (Yates corrected χ^2 test, $\chi^2 = 16.85$, $P \leq 0.00001$ and

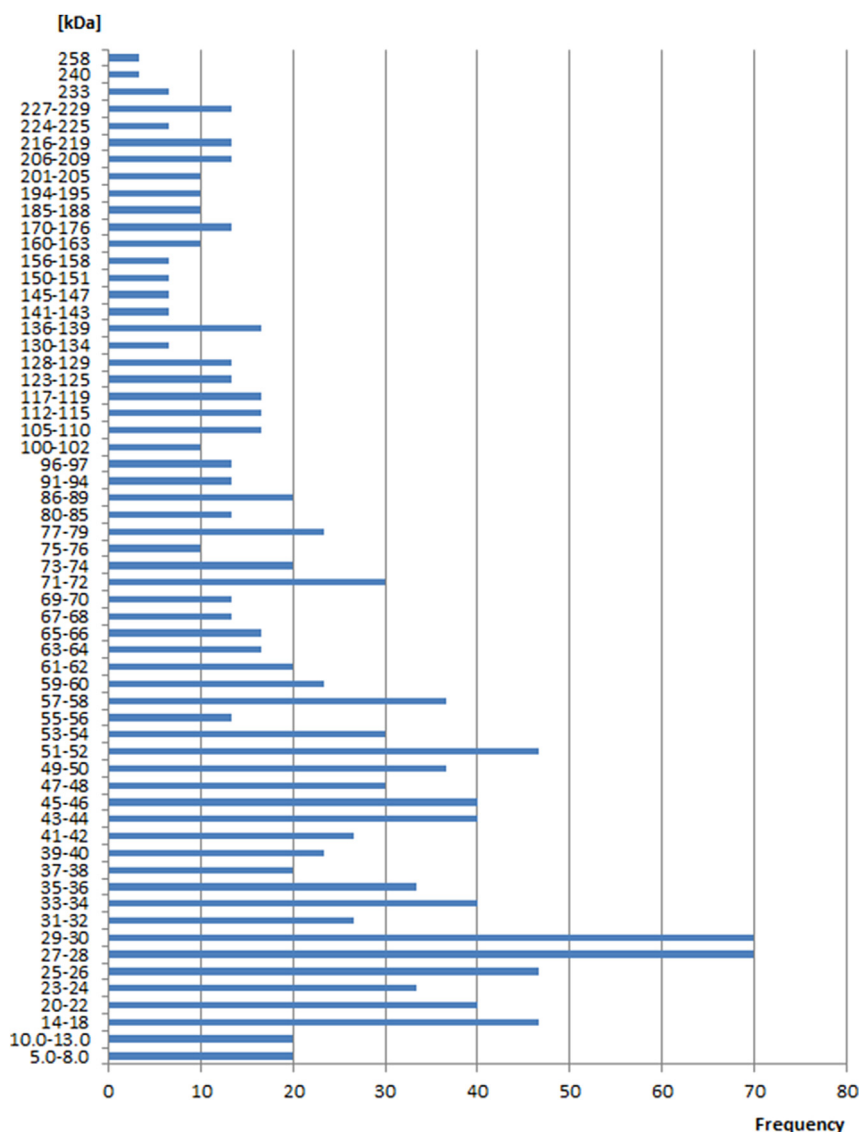


Fig. 4. Percent of sera giving positive reactions with antigens in the examined extracts of pupae *Sitophilus granarius*.

$\chi^2 = 12.50$, $P = 0.0004$, respectively) whereas the frequencies of positive reactions between pupae and larvae and between adults of both sexes were similar (Yates corrected χ^2 test, $\chi^2 = 0.81$ and 0.20 , respectively; $P > 0.05$).

4. Discussion

Store insects can be found in a wide variety of stored food products but also in farming environments. The most common storage insect species are sources of important allergens and can cause allergy among farmers, grain-storage workers, bakers, millers and other agriculture categories (Alvarez et al., 1996; Arlian, 2002; Astarita et al., 2001; Campbell et al., 2004; Jeebhay et al., 2005; Ravsbeck and Dueholm, 1990). Stored food products can also contain many other allergens like fungi, storage mites or other storage pests, and among them *S. granarius* (Alanko et al., 2000; Stejskal and Hubert, 2008).

The most important insects that can cause an occupational allergy are moths, psocids, cockroaches and beetles. There are known many species of beetles which are proved to be allergic occupational complaints like *S. granarius*, *Tenebrio molitor* and *Tribolium*

confusum (Alanko et al., 2000; Baldo and Panzani, 1988; Heyworth, 1999; Schultze-Werninghaus et al., 1991; Stejskal and Hubert, 2008). Some beetles are also important sources of allergens inside homes, particularly *Dermestes lardarius*, *Anthrenus verbasci*, *Attagenus pellio* or *Anobium punctatum* (Ahmed et al., 1981; Brito et al., 2002; Ramachandran et al., 1997).

Compared to storage mites, our knowledge about allergens of storage insects is still very poor. So far only a few studies have focused on this topic. Herling et al. (1995) have found that 54% of sera had elevated levels of IgE against the whole-body extracts of *S. granarius*. They also identified 23 different proteins (ranging 30–70 kDa) using crossed immunoelectrophoresis. Eleven proteins were capable of binding IgE. All individual sera reacted with at least four different proteins. Only two protein fractions bound IgE 88% and 100% of the patients and are considered to be the most important allergens from *S. granarius*. Alanko et al. (2000) demonstrated that allergies to two storage insects, *T. confusum* and *S. granarius*, were found in 5.4% of individuals tested through skin prick tests in specific serum IgE antibodies.

In the conducted studies it was shown that 100% of examined sera reacted with antigens of *S. granarius*. Moreover in the present

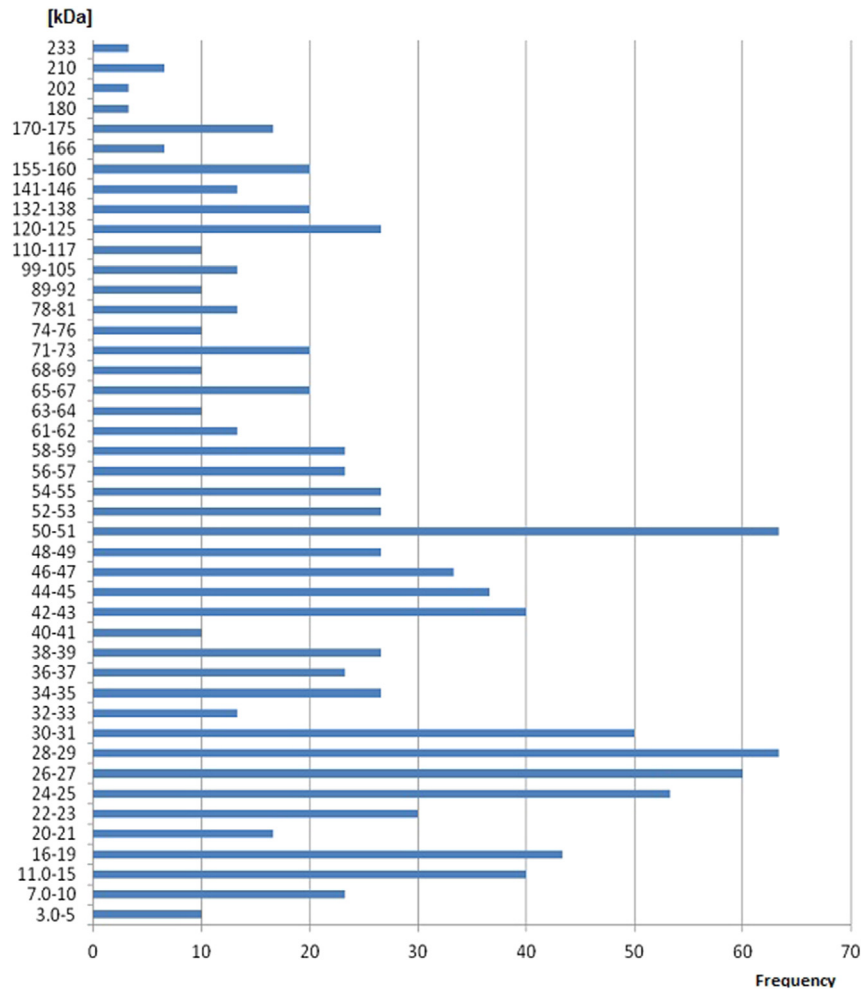


Fig. 5. Percent of sera giving positive reactions with antigens in the examined extracts of larvae *Sitophilus granarius*.

study demonstrated much more antigens giving positive reactions with IgE-antibodies in comparison to Herling's research. More than 33% of the sera showed IgE reactivity to protein fractions of 28–29 kDa and 52–53 kDa in extracts of females. More than 43% of the sera reacted with fractions located at 30–31 kDa, 49–50 kDa in extracts of males. 70% of the sera showed IgE reactivity to protein fractions of 27–28 kDa and 29–30 kDa in pupae. More than 63% of the tested sera reacted with fractions located at 28–29 kDa and 50–51 kDa in larvae. The studies show that all life stages are associated with protein fractions that interact with IgE. It shows that allergies can be associated with all life stages of this stored product pest.

5. Conclusions

The obtained results may indicate the potential existence of many protein fractions with allergenic properties in *S. granarius*, which may cause allergic reactions in occupationally exposed workers. It should be stressed that antigens from the particular developmental stages of the granary weevil have not been reported before.

References

- Ahmed, A.R., Moy, R., Barr, A.R., Price, Z., 1981. Carpet beetle dermatitis. *J. Am. Acad. Dermatol.* 5 (4), 428–432. [http://dx.doi.org/10.1016/S0190-9622\(81\)70104-X](http://dx.doi.org/10.1016/S0190-9622(81)70104-X).
- Alanko, K., Tuomi, T., Vanhanen, M., Pajari-Backas, M., Kanerva, L., Havu, K., Saarinen, K., Bruynzeel, D.P., 2000. Occupational IgE-mediated allergy to *Tribolium confusum* (confused flour beetle). *Allergy* 55 (9), 879–882.
- Alvarez, M.J., Tabar, A.I., Quirce, S., Olaguibel, J.M., Lizaso, M.T., Echechipia, S., Rodriguez, A., Garcia, B.E., 1996. Diversity of allergens causing occupational asthma among cereal workers as demonstrated by exposure procedures. *Clin. Exp. Allergy* 26 (2), 147–153. <http://dx.doi.org/10.1111/j.1365-2222.1996.tb00073.x>.
- Astarita, C., Gargano, D., Manguso, F., Romano, C., Montanaro, D., Pezzuto, F., Bonini, S., Altucci, P., Abbate, G., 2001. Epidemiology of allergic occupational diseases induced by *Tetranychus urticae* in greenhouse and open-field farmers living in a temperate climate area. *Allergy* 56 (12), 1157–1163.
- Arlian, L.G., 2002. Arthropod allergens and human health. *Annu. Rev. Entomol.* 47 (1), 395–433.
- Baldo, B.A., Panzani, R.C., 1988. Detection of IgE antibodies to a wide range of insect species in subjects with suspected inhalant allergies to insects. *Int. Arch. Allergy Immunol.* 85 (3), 278–287. <http://dx.doi.org/10.1159/000234518>.
- Brito, F.F., Mur, P., Barber, D., Lombardero, M., Galindo, P.A., Gomez, E., Borja, J., 2002. Occupational rhinoconjunctivitis and asthma in a wool worker caused by *Der-mestidae* spp. *Allergy* 57 (12), 1191–1194.
- Campbell, J.F., Arthur, F.H., Mullen, M.A., 2004. Insect management in food processing facilities. *Adv. Food Nutr. Res.* 48, 239–295. [http://dx.doi.org/10.1016/S1043-4526\(04\)48005-X](http://dx.doi.org/10.1016/S1043-4526(04)48005-X).
- Chambers, J., Collins, L.E., 2000. Optimisation and Validation of a Floor Trap for the Detection of Insect Pests in Empty Stores, in Bagged Stacks and on Flat Surfaces in the Cereal and Food Trades, pp. 1–28. Home Grown Cereals Authority Project-Report. No. 231.
- Cook, D.A., Armitage, D.M., 2002. Integrated pest management for stored grain in the UK incorporating diatomaceous earths to prevent surface infestations of insects and mites. *IOBC WPRS Bull.* 25 (3), 221–230.
- Giacinto, G.S., De Cristofaro, A., Rotundo, G., 2008. Behavioral responses of adult *Sitophilus granarius* to individual cereal volatiles. *J. Chem. Ecol.* 34 (4), 523–529.
- Herling, C., Svendsen, U.G., Schou, C., 1995. Identification of important allergenic proteins in extracts of the granary weevil (*Sitophilus granarius*). *Allergy* 50 (5),

- 441–446.
- Heyworth, M.F., 1999. Importance of insects in asthma. *J. Med. Entomol.* 36 (2), 131–132. <http://dx.doi.org/10.1093/jmedent/36.2.131>.
- Jeebhay, M.J., Baatjies, R., Lopata, A., 2005. Work-related respiratory allergy associated with sensitisation to storage pests and mites among grain-mill workers. (Allergies in the workplace). *Curr. Allergy Clin. Immunol.* 18 (2), 72–76.
- Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of the head of the bacteriophage T 4. *Nature* 227, 680–685.
- Ramachandran, S., Hern, J., Almeyda, J., Main, J., Patel, K.S., 1997. Contact dermatitis with cervical lymphadenopathy following exposure to the hide beetle, *Dermestes peruvianus*. *Br. J. Dermatol.* 136 (6), 943–945. <http://dx.doi.org/10.1046/j.1365-2133.1997.01828.x>.
- Revsbech, P., Dueholm, M., 1990. Storage mite allergy among bakers. *Allergy* 45, 204–208. <http://dx.doi.org/10.1111/j.1398-9995.1990.tb00484.x>.
- Schultze-Werninghaus, G., Zachgo, W., Rotermund, H., Wiewrodt, R., Merget, R., Wahl, R., Burow, G., Zur Strassen, R., 1991. *Tribolium confusum* (confused flour beetle, rice flour beetle)—an occupational allergen in bakers: demonstration of IgE antibodies. *Int. Arch. Allergy Immunol.* 94 (1–4), 371–372.
- Stejskal, V., Hubert, J., 2008. Risk of occupational allergy to stored grain arthropods and false pest-risk perception in Czech grain stores. *Ann. Agric. Environ. Med.* 15 (1), 29–35.
- Towbin, H., Staehelin, T., Gordon, J., 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci.* 76, 4350–4354.
- Woodbury, N., 2008. Infanticide avoidance by the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae): the role of harbourage markers, oviposition markers, and egg-plugs. *J. Insect Behav.* 21 (2), 55–62. <http://dx.doi.org/10.1007/s10905-007-9105-0>.