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Prevalence of sensitization to extracts from particular life stages of the saw-toothed grain beetle (*Oryzaephilus surinamensis*) in citizens of selected suburban areas of Southern Poland

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ABSTRACT

Many species of insects are agricultural pests which cause not only economic losses but also allergies in humans. The subject of this study was to identify important antigens from the saw-toothed grain beetle – *Oryzaephilus surinamensis* [OS]. Sera of 30 patients from a suburban population of Upper Silesia (Southern Poland) were tested for the presence of IgE antibodies to antigens from particular active life stages of OS (larvae, pupae and adults of both sexes). The collected proteins were fractionated by SDS PAGE and identified by Western blot. The patient's antibodies against particular antigens were identified using anti-human anti-IgE monoclonal antibody. The conducted studies showed the existence of many protein fractions for each life stage of OS which give positive reactions with IgE antibodies. The largest number of allergenic potential fractions was shown in females (23 protein fractions) and pupae (22 protein fractions) while smaller amount was shown in larvae (18 protein fractions) and males (14 protein fractions). Majority of the sera (25/30) showed positive reactions to protein fractions 25–29 kDa and 30–34 kDa from pupae of OS. The obtained results may indicate the existence of many protein fractions with an allergenic properties in OS. It also should be stressed that all life stages of this storage insect may provoke allergic reactions in exposed subjects.

1. Introduction

Many species of insects can be harmful for a human and human's health. There are, for instance, many insects which are agricultural pests and damage different types of stored foodstuffs. Many of those pests can cause allergies in humans. Among this group of organisms is the largest and most diverse insect order, Coleoptera, commonly known as beetles. Beetles can cause sensitize reactions in humans, especially in the working environment (Auerswald and Lopata, 2005). Exposure to grain dust may cause a large number of allergy symptoms including conjunctivitis, rhinitis, dermatitis and asthma (Jeebhay et al., 2005). This article presents research conducted on the insect which can cause such reactions although the knowledge about its allergens is still very poor.

Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae) is a very common and destructive insect occurring in the warehouses and feeding on the stored products. This species is a cosmopolitan pest, which can be found in the great variety of products and habitats. It is very common in mills, fodder storages and shops (Kłyś and Przystupińska, 2015; Laszczak-Dawid et al., 2008; Sinha and Watters, 1985; Trematerra and Sciarretta, 2004). *O. surinamensis* is also one of the most frequently found pests of grain and cereal products in Poland. The beetle feeds mainly on ground cereal products, but it can also feeds on nuts, dried fruit and seeds, and less often on processed foodstuffs (Kłyś and Przystupińska, 2015; Madkour et al., 2013; Sandner, 1990).

2. Materials and methods

Oryzaephilus surinamensis was bred in the laboratory conditions. The whole body extracts were prepared from four life stages of the beetle – larvae, pupae, males and females. These life stages 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

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Silesia (South Poland) were collected in the Health Care Center and examined. They were a waste material after routine laboratory diagnostic control. The research were 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.

First, insect bodies were homogenized in Sample Buffer (SB) and denatured at 100 °C for 5 min. Next, the concentrations of proteins were measured by spectrophotometry on the nano-spectrophotometer PEARL (Implen, Germany). The SDS-PAGE was performed according to Laemmli (1970) with some modifications. It was made using a mini-Protean II system (BioRad, USA). The electrophoresis was performed at 100 V for 90 min. 12% separating gels were used. Gels were electrotransferred according to modified method of Towbin et al. (1979) using a Mini-Transblot Cell (BioRad, USA). Electrotransfer was carried out at 150 mA for 1 h. 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). Then 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. Next, the 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).

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

Practically, positive reactions to protein fractions of. *O. surinamensis* were shown in all collected sera, although western blot analysis revealed differences in reactivity of the examined sera with particular fractioned life stages (Figs. 2–5).

The largest number of allergenic potential fractions was observed in females and pupae, while smaller number was detected in larvae and males. Among the four life stages, the largest percentage of the patients reacted to two fractions from pupae located at 25–29 kDa and 30–34 kDa (83.3% each). There was no other fraction from any other life stage exceeds this frequency.

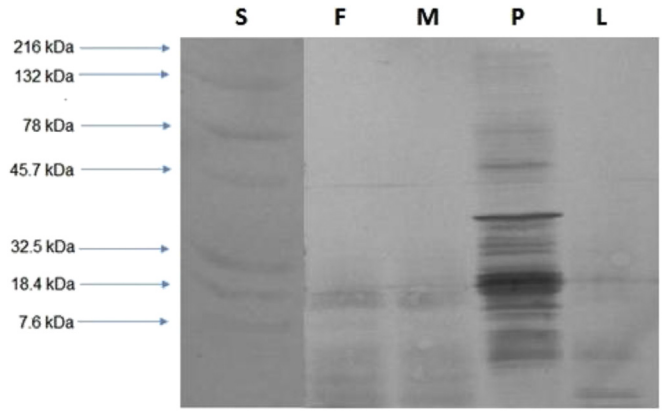

Fig. 1. The IgE response of the exemplary patient against protein fractions of *Oryzaephilus surinamensis*. Explanations: S – molecular weight standard (BioRad USA); F – protein fractions of females; M – protein fraction of males; P – protein fractions of pupae; L – protein fractions of larvae.

In case of pupae the largest amount of sera reacted with protein fractions located at 25–29 kDa (83.3%), 30–34 kDa (83.3%), 35–39 kDa (73.3%), 40–44 kDa (63.3%) and 45–49 kDa (73.3%) (Fig. 4). The protein fractions of females located at 26–29 kDa (30%), 30–35 kDa (33.3%) and 40–45 kDa (46.6%) showed the most frequent positive reactions (Fig. 2). The most sera reacted with protein fractions located at 24–28 kDa (30%), 30–33 kDa (30%) and 45–49 kDa (33.3%) in males and at 30–34 kDa (26.6%) and 40–44 kDa (30%) in larvae of *O. surinamensis* (Figs. 3 and 5).

The smallest percentage of sera reacted with fractions located at 89, 100 and 132 kDa in females, 93 kDa in males, 3, 89, 96, 118, 140 and 204 kDa in pupae and 3, 81 and 116 kDa in larvae (Figs. 2–5).

Examined sera reacted most frequently with protein fractions about 45 kDa in all tested life stages. Comparison of protein fractions located at 45 kDa shows that sensitization to pupae extracts was statistically more frequent compared to larval extracts (Yates corrected χ^2 test, $\chi^2 = 35.3$; $P \leq 0.00001$), females (Yates corrected χ^2 test, $\chi^2 = 13.2$; $P < 0.0005$) and males (Yates corrected χ^2 test, $\chi^2 = 30.53$; $P \leq 0.00001$). Moreover, sensitization to females was significantly more frequent than to larvae (Yates corrected χ^2 test, $\chi^2 = 5.41$; $P = 0.02$) However, frequencies of positive reactions for the 45 kDa protein fractions between larvae and males and between both adults were not significantly different (Yates corrected χ^2 test, $\chi^2 = 0.09$ and 3.5, $P > 0.5$ and $P > 0.05$, respectively).

4. Discussion

Stejskal and Hubert (2008) have found that 83% of collected in Czech Republic grain samples were infested by over 1 million pest-arthropod individuals. Among them the most frequent and abundant were mites, followed by psocids and beetles. These groups of arthropods play a role as allergenic contaminators of stored food. It is a risk not only for people working in places connected with agriculture but also for people consuming infested products.

The main classes of insects that cause occupational allergies are cockroaches and beetles (Jeebhay et al., 2005). There are known many species of beetles which are proved to be the source of important allergens, including *Sitophilus 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).

Compared to many other species of storage insects or storage mites, our knowledge about allergens of *O. surinamensis* is still very poor. So far there have not been other research focused on examined species. In Jeebhay et al, (2005) research the patterns of sensitization in a group of grain mill workers demonstrated that storage mites (26%) and cockroaches (22%) caused IgE reactivity. The strongest IgE response among the storage mites was produced by *Blomia tropicalis*. Insects such as beetles *Sitophilus granarius* (16%) and *Tenebrio molitor* (13%) produced a lower proportion of sensitised individuals. Armentia et al. (1997) observed among Spanish grain mill workers a high prevalence of IgE reactivity to *Tenebrio molitor* (50%) and less to storage mites (38%) and cockroaches (36%). Herling et al. (1995) have found that 54% of sera had elevated levels of IgE reactivity to *Sitophilus granarius*.

In our previous studies (Jakubas-Zawalska et al., 2016) on *S. granarius* it was shown that 100% of examined sera reacted with antigens of this insect. There also were shown differences in reactivity of the sera with particular life stages. It has been found that among the examined life stages not only the largest number of protein fractions but also the largest percentage of the patients