Pak. J. Bot., 49(4): 1497-1500, 2017.

ANALYSIS OF PROTEIN PROFILE AND POLLEN MORPHOLOGY OF *GUAIACUM OFFICINALE* LINN.

SHAISTA ZEB[1], ANJUM PERVEEN[2*] AND MUNEEBA KHAN[2]

[1]*Department of Botany, University of Karachi, Pakistan*
[2]*Centre for Plant Conservation, University of Karachi, Pakistan*
[*]*Correspondence author's email:anjum_tahir@hotmail.com*

Abstract

Asthma and allergic rhinitis is triggered by the pollen of trees, grasses and weeds. *Guaiacum officinale* L. tree is widely cultivated along with the road side. This species was selected to check its allergenic role.Pollen morphology of *Guaiacum officinale* was examined by Light microsco4pe (LM) and Scanning electron microscope (SEM). Pollen grains of *Guaiacum officinale* were prolate shape, having tricolpate aperture, and rugulate tectum. Pollen protein concentration of *G. officinale* was determined by Bradford's assay and qualitative protein analysis of pollen was done by SDS–PAGE(Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis).Total protein content in the pollen extract was 24.28mg/g of pollen. The SDS-PAGE pollen grains protein analysis showed 07 different protein bands. The molecular weight of separated proteins ranged from 25 to 65kDa. Biochemical analysis of *G. officinale* pollen grains revealed the presence of low molecular weight proteins therefore it is strongly suggested that this species must be considered as a potent allergy causing species. This research would help for the proper diagnosis and treatment of the bronchial allergy suffering patients.

Key words: Pollen allergy; Bronchial asthama; SDS-PAGE analysis; Allergenic proteins.

Introduction

Airborne pollen allergy is a major problem for a significant percentage of people. Therefore the knowledge about the types of pollen grain present in the air and their relation to allergy symptoms is very important for allergy suffering individuals. The most common symptoms in people allergic to pollen include inflammation of the nasal mucosa, characterized by sneezing, nasal obstruction, itchy and runny nose and eyes. Hay fever occurs due to inhalation of high concentration of pollen (Wilson *et al*., 1973).Pollen grains of tree, weeds and grasses are the main causative agent of respiratory tract sensitivity as shown in reports of allergy to pollen throughout the world (Garcia-Ortega*et al*., 1992; Arnon *et al.*, 1998; Singh & Kumar, 2003; Dursun *et al*., 2008; Can *et al*., 2010).

Pollen allergy is caused by proteins, glycoprotein (Chanda, 1994). Pollen grains contain different types of proteins have the capability to interact with the human immune system and to cause allergic reactions. Biochemical characteristics of allergenic proteins contribute to this interaction, including localization of allergens contained by pollen grains (Knox &Harrison, 1970; Singh *et al*., 1991; Arnon & Regenmortel, 1992; Vrtala *et al*., 1993). The biochemical and immunological standardization of antigenic and allergenic components is being emphasized for definite identification and controlling of allergic diseases all over the world (Karmakar & Chatterjee, 1992). The detection and characterization of allergy causing proteins or glycoprotein is very demanding task for agrobiologists (Cresti &Tiezzi, 1992). These allergenic proteins weights generally in the range of 10–70 kDa and are present in intine, exine and different parts of the pollen including ground cytoplasm (Knox &Suphioglu, 1996; Puc, 2003; Verdino, 2006; Chapman *et al*., 2007).

Pollen grains inhalation may cause allergy attack in hypersensitive individuals. Airborne pollen concentrations fluctuate in the different seasons depending upon the flowering period, geography and climatic conditions. Identification of pollen types and their occurrence pattern is significant for clinicians and allergy patients for proper diagnosis of allergy causing agent (D'Amato & Spieksma, 1991; Garcia-Mozo *et al.*, 2006). Several aerobiological investigations were carried out on this subject in number of cities and pollen calendar of studied area were prepared (Nilsson & Gothard, 1982; Emberlin *et al*., 1990; Recio *et al*., 1998; Latorre, 1999; Dopazo *et al*., 2000; Guvensen & Ozturk, 2002; Boral *et al*., 2004; Ayvaz *et al*., 2008; Abu-Dieyeh *et al*., 2012). Similar studies have been performed in Pakistan particularly in Sindh province (Perveen *et al*., 2012; 2014; 2015).

Guaiacum officinale belong to family Zygophyllaceae usually known as "Rough bark Lignum-vitae" very common entomophilous small tree. It is planted abundantly on road sides, streets and in parks as ornamental tree in different areas of Sindh. It flowering period remains from March to October. *Guaiacum officinale* pollen grains have been recorded in the atmosphere from various parts of Sindh (Waqar *et al*., 2010; Perveen *et al*., 2014).

Less information is available about pollen allergy by *Guaiacum officinale* tree. So, pollen of this tree were selected for the aerobiological investigation. The aim of this research work is to identify *Guaiacum officinale* pollen protein concentration and to check the presence of low molecular weight proteins which might become to cause of allergy in hypertensive individuals.

Material and Method

Pollen collection: Mature flowers of *Guaiacum officinale* were collected during the flowering season. Anthers were separated from the flowers. The anthers were crushed gently after drying and pollen grains were sieved through 100μm and 200μm mesh size sieves. Pollen purity was checked by light microscopy. Pollen were treated with acetone and dried. Defatted pollen grains were stored in glass vials at -4°C.

Pollen morphological studies: Pollen of *Guaiacum officinale* was prepared for morphological studies by the standard acetolysis method as described by Erdtman, (1952). Light microscopy was done after making permanent slides and for scanning electron microscopy (SEM) purified pollen grains were shifted on to a metallic stub having double adhesive tape. Pollen grains were coated with gold in a sputtering chamber (Ion-sputter JFC-1100) S.E.M was carried out by microscope (JSM-6380A).

Different readings were taken for pollen morphology viz., polar length; equatorial diameter; exine ornamentation; exine thickness; aperture type and colpus length.

Pollen protein extraction and estimation: Pollen grains protein was extracted in phosphate buffer saline or PBS (pH 7.4) at 04ºC. The proteins concentration estimation of *Guaiacum officinale* pollen grains done by Bradford method (1976). Readings were taken at 595nm wavelength using spectrophotometer.

Gel Electrophoresis (SDS–PAGE): Protein profiling was carried out by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) according the methods of Laemmli (1970) with slight modification. 12% resolving gel and 4% stacking gel was used for the separation of protein bands. 20µl of the protein sample was loaded. Electrophoresis was carried out at a constant voltage of 100 Volts. Silver stain was used to detect protein bands in gel. Distaining of the gel was done to remove excess of dye by using distaining solution.

Results

Microscopic characterization of *Guaiacum* pollen: P/E ratio 180; shape prolate; aperture type tricolpate; polar axis 15.6µm (18.2µm) 23.4µm; equatorial diameter 7.8µm (10.66µm) 13 µm; colpi length10.4µm (12.67µm) 14.3 µm; exine 1.3 µm; sexine is thicker than nexine. Tectum rugulate (Fig. 1).

Protein analysis

Protein estimation by Bradford assay: The total protein content of the pollen extracts was determined by the Bradford protein assay. Bovine serum albumin (BSA) for making standard curve. The total protein content of pollen grain extract in PBS was 24.28mg/g of dry weight of pollen.

Protein profiling by SDS-PAGE: A total of 07 protein bands were detected in the pollen grains by SDS–PAGE. The molecular weight of resolved protein bands ranged from 25 to 65kDa.The protein bands with different molecular weight in the pollen grains sample were. 25 kDa, 27 kDa, 29kDa, 33 kDa, 35 kDa, 51 kDa and 65 kDa. It was noticed that in *Guaiacum officinale* pollen grains separated proteins molecular weight were less than70 kDa (Fig. 2).

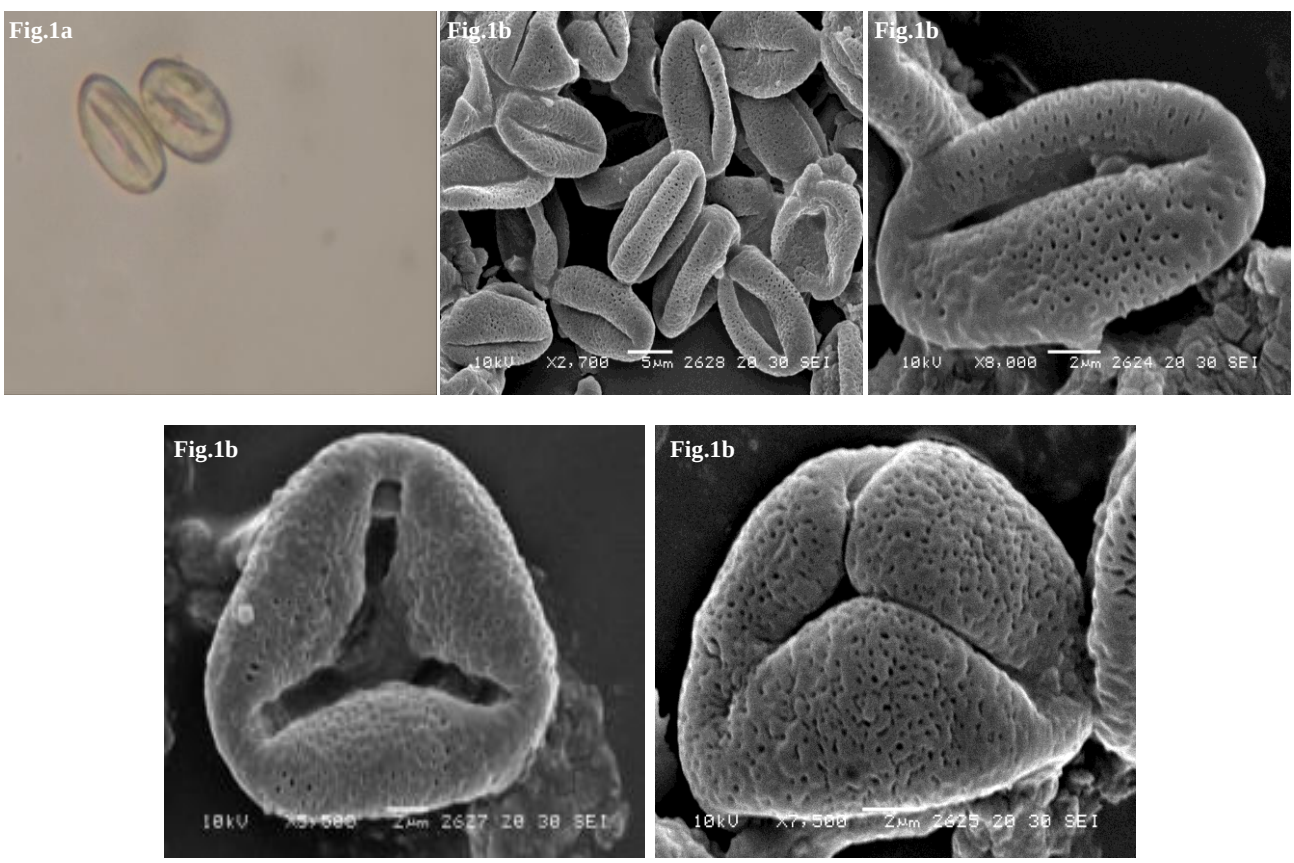

Fig. 1b

Fig. 1. Pollen grains of *Guaiacum officinale*; Light microscopic photomicrographs(1a), Scanning electron photo-micrograph (1b)

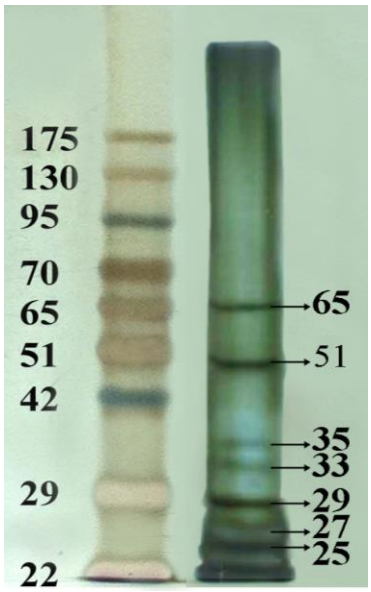

Fig. 2. Protein profiling of *Guaiacum officinale* pollen by SDS-PAGE (Molecular Weight of bands were assigned on the basis of comparison with molecular weight marker).

Discussion

Guaiacum officinale L., is a very common tree along road side for its thick crown of close growing foliage and in parks due to lilac colored flowers. Although the pollination of *Guaiacum officinale*is entemophilous plant but it releases a considerable number of pollen grains in the air during its flowering season from March to October (Ghafoor, 1974). Agashe (1989) reported that insect pollinated plant may also become the cause of hay fever. *Guaiacum* pollen grains have been reported from the atmosphere from Tandojam, Khairpur and in Karachi (Waqar *et al*., 2010, Perveen*et al.,* 2012; 2014; 2015). A number of tree pollen grains are considered to be important part of the spectrum of allergy stimulate agent from the local flora (Eriksson *et al*., 1984; Rawat *et al*., 2000).

It is generally believed that allergic reaction inhuman beings caused by wind pollinated common widespread species that produce pollen in high quantity (Behrendt & Becker, 2001; Culley *et al*., 2002). In contrast common recognition that entomophilous pollen does not become airborne however there are some entomophilous pollen detected in aeropalynological survey (Mandal &Chanda, 1981; Tilak, 1984). Clinical analysis of certain entomophilous pollen showed allergic reactions in sensitive person even inadequate concentration present in the air (Tilak, 1984).

Protein profile of *Guaiacum officinale* pollen grains was carried out by SDS-PAGE. Gel Electrophoresis (SDS-PAGE) is a frequently used tool for proteins analysis and purification. A total of 07 protein bands were detected in the *Guaiacum* pollen grains. Allergenic pollen contains a set of a number of allergenic proteins which are responsible to cause allergy. A single pollen type contains several allergens. 11 groups of allergens have been identified in grass pollen (Andersson & Lidholm, 2003; Petersen *et al*., 2006). It was noteworthy that 7 protein bands were less than70 KDa were detected in *Guaiacum* pollen extract which could be considered as allergenic protein bands. Low molecular weight proteins were identified as major cause of allergy for example in Paper Mulberry 33 kDa and 40 kDa molecular weight proteins were confirmed for causing allergy (Aslam *et al*., 2015). Several investigations reported that pollen proteins of *Prosopis juliflora* (Mesquite tree) with molecular weight of 45 kDa and 66kDa are allergenic (Dhyani *et al*., 2008). *Acacia* pollen extract by SDS-PAGE obtained several bands with molecular weights ranging from 12kDa to 85kDa and among those six bands viz., of 85, 66, 39, 45, 28, 23, and 15kDa showed to reduce IgE antibody (Shamsbiranvand *et al*., 2014).

Our study strongly suggested that *Guaiacum officinale* pollen grains are allergenically important. Local people must be recognize the blooming periods of this plant to avoid allergic reaction from this plant

Conclusion

The outcome of the investigation will provide a platform for further isolation and molecular characterization of their main allergenic proteins, which is essential for the treatment of allergic patients sensitive to *Guaiacum officinale* pollen grain.

Reference

Abu-Dieyeh., M.H. and Y.S. Ratrout. 2012. Seasonal variation of airborne pollen grains in the atmosphere of Zarqa area, Jordan. *Aerobiologia*, 28: 527-539.

Agashe, S.N. 1989. Airborne entomophilous pollen-potential source of allergy. In: (Ed.): Tilak, S.T. Recent Researches in Ecology. *Environment and Pollution*, 153-157.

Andersson, K. and J. Lidholm. 2003. Characteristics and immunobiology of grass pollen allergens. *Int. Arch. Allergy Immunol.*, 130: 87-107.

Arnon, G., R.C. Chohen and W. Yoav. 1998. Allergic response to pollen of ornamental plants: high incidence in general atopic population and especially among flower growers. *J. Allergy Clin. Immunol.*, 102: 210-214.

Arnon, R. and M.H.V. Regenmortel. 1992. Structural basis of antigenic specificity and design of new vaccines. *FASEB J.*, 6:3265-3274.

Aslam, M.S., T. Khalid, I. Gull, Z. Abbas and M.A. Athar. 2015. Identification of major allergens of Paper Mulberry (*Broussonetia papyrifera*) pollens and purification of novel 40 kDa allergen protein. *Curr. Allergy Clin. Im.*, 28(1): 36-41.

Ayvaz, A., A. Baki and C. Dogan. 2008. Seasonal distributions of aeroallergens in the atmosphere of Trabzon, Turkey. *Asthma Allergy Immunol.*, 6(1): 11-16.

Behrendt, H. and W.M. Becker. 2001. Localization, release and bioavailability of pollen allergens: The influence of environmental factors. *Curr. Opinion Immunol.*, 13: 709-715.

Boral, D., S. Chatterjee and K. Bhattacharya. 2004. The occurrence and allergising potential of airborne pollen in West Bengal, India. *Ann. Agric. Environ. Med.*, 11: 45-52.

Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248-254.

Can, I.H., A. Islam, D.S. Karasoy and E.F. Samim. 2010. Does regional pollen load affect the prevalence of clinical allergy to those pollen groups? *J. Laryngol. Otol.*, 124(3): 297-301.

Chanda, S. 1994. Pollen Grains as Aeroallergens: Morphological, Biological and Chemical Approach. In: (Ed.): Agashe, S.N. Recent Trends in Aerobiology. *Allergy and Immunology*, 85-92.

Chapman, M.D., A. Pomes, H. Breiteneder and F. Ferreira. 2007. Nomenclature and structural biology of allergens. *J. Allergy Clin. Immunol.*, 119: 414-420.

Cresti, M. and A. Tiezzi (Rd). 1992. Sexual Plant Reproduction. *Springer Verlag Berlin.*, 203-217.

Culley, T.M., S.G. Weller and A.K. Sakai. 2002. The evolution of wind pollination in angiosperms. *Tr. Ecol. Evol.*, 17361-369.

D'Amato, G. and F.T. Spieksma. 1990. Allergenic pollen in Europe. *Grana*, 30: 67-70.

Dhyani, A., B.P. Singh, N. Arora, V.K. Jain and S. Sridhara. 2008. A clinically relevant major cross-reactive allergen from mesquite tree pollen. *Eur. J. Clin. Invest.*, 38(10): 774-781.

Dopazo, A., V. Jato and M.J. Aira. 2000. Allergenic pollen types in the atmosphere of Santiago de Compostela (NW Spain): A pollen calendar for the last six years. *Bot. Helv.*, 110: 51-60.

Dursun, A.B., G.E. Celik, S. Alan, N.M. Pinar, D. Mungan and Z. Misirligil. 2008. Regional pollen load: Effect on sensitization and clinical presentation of seasonal allergic rhinitis in patients living in Ankara, Turkey. *Allergol Immunopathol (Madr)*, 36(6): 371-378.

Emberlin, J., J. Norris-Hill and R. Bryant. 1990. A calendar for tree pollen in London. *Grana.*, 29: 301-309.

Erdtman, G. 1952. Pollen morphology and plant taxonomy angiosperm. *Chronica botanica* Co., Walthman, Massachusettes.

Eriksson, N.E., J.A. Wihl, H. Arrendal and S.O. Strandhede. 1984. Tree pollen allergy II. Sensitization to various tree pollen allergens in Sweden-a multicentre study. *Allergy*, 39: 610-617.

Garcia-Mozo, H., R.P. Badia, F.F. Gonzalez and C. Galan. 2006. Airborne pollen sampling in Toledo, Central Spain. *Aerobiologia*, 22: 55-66.

Garcia-Ortega, P., J. Mortnez, A. Martiniz, R. Palacios, J. Belmonte and J. Richart. 1992. *Mercularis annua* pollen: A new source of allergy sensitization and respiratory disease. *J. Allergy Clin. Immunol.*, 89: 987-993.

Ghafoor, A. 1974. Zygophyllaceae. In: Flora of Pakistan. (Eds.): Nasir, E. and S.I. Ali. 76: 1-35, Karachi University, Pakistan.

Guvensen A. and M. Ozturk. 2002. Airborne pollen calendar of Buca-Izmir, Turkey. *Aerobiologia*, 18: 229-237.

Karmakar, P.R and B.P.Chatterjee.1992. Chemical modification studies on *Cocos nucifera* pollen allergens. *J. Biosci.*, 17(4):441-52.

Knox, B. and C. Suphioglu. 1996. Environmental and molecular biology of pollen allergens. *Trend in Plant Scienc.*, 5:156-164.

Knox, R.B. and J.H. Harrison. 1970. Pollen-wall proteins: Localization and enzymic activity. *J. Cell Sci.*, 6: 1-27.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227(5259): 680-685.

Latorre, F. 1999. Differences between airborne pollen and flowering phenology of urban trees with reference to production, dispersal and interannual climate variability. *Aerobiologia*, 15: 131-141.

Mandal, S. and S. Chanda. 1981. Aeroallergens of West Bengal in the context of environmental pollution and respiratory allergy. *Biol. Mem*., 6: 1-61.

Nilsson, S. and J.P. Gothard. 1982. Pollen calendar for Huddinge (Sweden), 1973–1980. *Grana*, 21: 183-185.

Perveen, A., M. Khan and S. Zeb. 2012. Identification and quantification of airborne pollen from Hyderabad: Tando-Jam, Sindh. *Pak. J. Bot.*, 44 (5): 1755-1762.

Perveen, A., M. Khan, S. Zeb and A. Imam. 2015. Impact and correlation of environmental conditions on pollen counts in Karachi, Pakistan. *Iran J. Allergy Asthma Immunology*, 14(1): 83-90.

Perveen, A., S. Zeb and M. Khan. 2014. Seasonal fluctuations of airborne pollen grains count and its correlation with climatic factors from Khairpur; Sindh, Pakistan. *Pak. J. Bot.*, 46(1): 299-306.

Petersen, A., R. Suck, , B. Lindner, D. Georgieva, M. Ernst, H. Notbohm, D. Wicklein, O. Cromwell and W.M. Becker. 2006. Phl p3: Structural and immunological characterization of a major allergen of timothy grass pollen. *Clin. Exp. Allergy*, 36(6): 840-849.

Puc, M. 2003. Characterization of pollen allergens. *Ann. Agr. Environ. Med.*, 10(2): 143-149.

Rawat, A., A. Singh, S.N. Gaur, L. Kumar, I. Roy, P. Ravindran and A. B. Singh. 2000. Clinical and immunologic evaluation of *Cedrus deodara* pollen: a new allergen from India. *Allergy*, 55: 620-626.

Recio, M., B. Cabezudo, M.M. Trigo and F.J. Toro. 1998. Pollen calendar of Malaga (Southern Spain), 1991-1995. *Aerobiologia*, 14: 101-108.

Shamsbiranvand, M.H., A. Khodadadi, M.A. Assarehzadegan, S.H. Borsi and A. Amini. 2014. Immunochemical characterization of Acacia pollen allergens and evaluation of cross-reactivity pattern with the common allergenic pollens. *J. Allergy*, 56-62.

Singh, A.B. and P. Kumar. 2003. Aeroallergens in clinical practice of allergy in India. An over view. *Ann. Agr. Environ. Med.*, 10(2): 131-136.

Singh, M.B., T. Hough, P. Theerakulpisut, A. Avjioglu, S. Davies, P.M. Smith, P. Taylor, R.J. Simpson, L.D. Ward and J.M. Cluskey. 1991. Isolation of cDNA encoding a newly identified major allergenic protein of rye-grass pollen: Intracellular targeting to the amyloplast. *Proc. Natl. Acad. Sci. U. S. A.*, 88(4): 1384-1388.

Tilak, S.T. 1984. Airborne entomophilous pollen. *J. Pl. Nat.*, 1(1): 45-50.

Verdino, P. 2006. Structural characterization of pollen allergens. *Clin. Rev. Allergy Immunol.*, 30: 73-95.

Vrtala, S., M. Grote, M. Duchene, R. Van Ree, D. Kraft, O. Scheiner and R. Valenta. 1993. Properties of tree and grass pollen allergens: reinvestigation of the linkage between solubility and allergenicity. *Int. Arch. Allergy Immunol.*, 102(2): 160-169.

Waqar, M.A., S.M. Hasnain and M. Khan. 2010. Airborne pollen survey in Karachi: A coastal city in Sindh province of Pakistan. *Indian J. Aerobiol.*, 23: 7-17.

Wilson, A.F., H.S. Novey, R.A. Berke and E.L. Surprenant. 1973. Deposition of inhaled pollen and pollen extract in human airways. *N. Engl. J. Med.*, 228: 1056-1058.

(Received for publication 25 April 2016)