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Editorial

How to interpret pollen counts

POLLEN SAMPLERS

Blackley[1] was the first investigator to describe and report, back in 1873, that hay fever (in his own wording, *"Catarrhus æstivus"*) was caused by the inhalation of grass pollens. This British physician, a true pioneer ahead of his time, not only observed and demonstrated the utility of skin tests in the diagnosis of this condition, but he also developed several types of pollen sampling traps. One of the most efficient ones among them was a simple petroleum jelly-smeared glass slide mounted vertically on a weather vane (Fig. 1). Every 24 hours, Blackley collected the slide and examined under a light microscope the various types of pollens that had become impacted, observing that his hay fever patients worsened only during the period when the grass pollen counts in Manchester were high (June and July).

In 1946, the US physician Durham[2] proposed his own standardised gravimetric sampling method, which was eventually accepted in most countries (Fig. 2). The fundament of this method was a horizontally mounted slide covered with an impaction medium to which pollens adhered and became impacted through gravimetric deposition. However, the collection efficiency of this method was good only for particles larger than 20 µm in size, and it was bad for spores and for small-sized pollens (e.g., *Castanea*, Urticaceae), which often simply flew over the slide without becoming impacted. The wind speed was also a serious disadvantage: the deposition of pollens became increasingly poorer as the wind speed increased.

W. A. Perkins later developed the first rotating rod (Rotorod®) collector, which was then designed for intermittent use by Metrónic in 1957 and which is still widely used in the USA (Fig. 3)[3]. This collector consists of two slender rods covered with an impaction medium that rotate in the same manner as an electric fan, but intermittently. The disadvantage of this method is its poor sampling efficiency for particles smaller than 10 µm in size; furthermore, its sampling ability decreases over time as increasing numbers of particles become impacted onto the sampler arms.

J. M. Hirst[4], in 1952, developed the first aspiration sampler (Fig. 4). This device consisted of an air admission chamber with a 10-l/min flow that led the airflow through a 14 x 2-mm slit, which is always oriented to windward as the whole device is mounted on a weather vane. The airflow coming out of the slit was directed onto a vertical, petroleum jelly-smeared slide, which moved relative to the slit at a rate of 2 mm/h (48 mm = 24 hours of sampling). The advantage of the Hirst and Rotorod® (volumetric) samplers as compared to the (gravimetric) Durham one is that the

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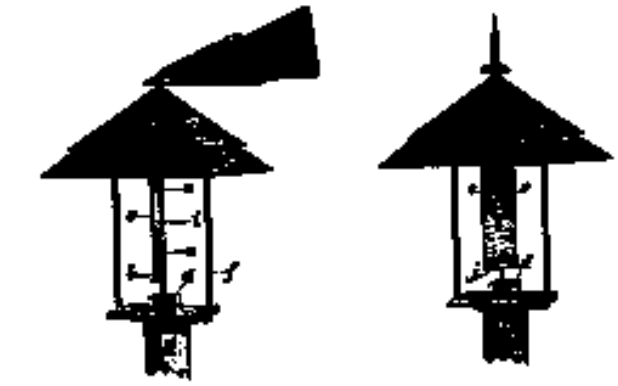

Fig. 1. Original drawing by Blackley representing, in frontal and lateral views, the pollen collector he designed for his investigations. From Blackley CH. Experimental Researches on the Nature and Causes of Catarrhus Aestivus. London, Bailliére Tyndall and Cox, 1873[1].

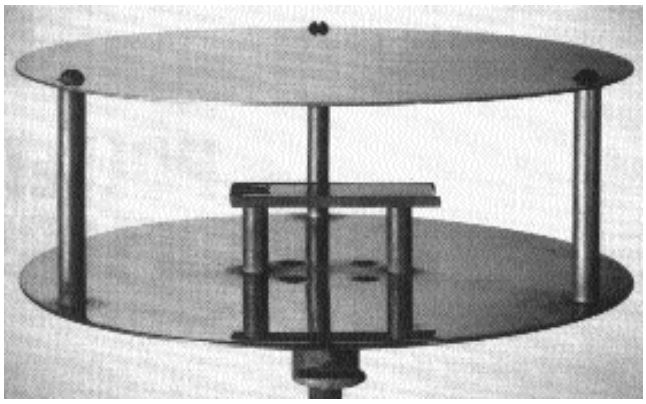

Fig. 2. The Durham collector. A glass slide smeared with petroleum jelly is placed on the horizontal support between two protecting plates.

Fig. 3. Rotorod® intermittent sampler. The two sampling arms are folded onto a support bar; when the device starts rotating the sampling arms open vertically downwards and expose the impaction rods.

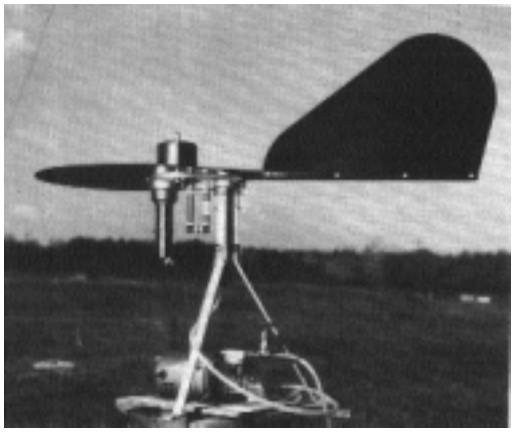

Fig. 4. The Hirst Spore Trap®. The particles are aspirated through a small (14 x 2 mm) slit. The impaction slide is placed within the cylinder very close to the slit, and the cylinder is vertically moved at a 2-mm/h rate by a clockwork mechanism.

volume of air that is sampled is known exactly. The advantages of the Hirst device as compared to the Rotorod® one are its higher sampling efficiency for particles smaller than 10 µm in size and the fact that its sampling ability does not decrease over time[5].

In the late ‘70s, the Burkard company developed the "Burkard Seven-Day Volumetric Spore-Trap®" (Fig. 5), a sampler that is fully based on the Hirst principle but with the added advantage that the impaction occurs onto a 345-mm band instead of onto a 76-mm slide, rendering possible an uninterrupted sampling period of seven days instead of only 24 hours in the Hirst device (as both the Hirst and the Burkard devices were registered under the name "Spore-Trap", this designation will be used henceforward even though they not only sample spores).

The Burkard spore-trap is the one most widely used in most sampling networks throughout the world, and it is also the one used by the Sampling Network of the Spanish Society of Allergy and Clinical Immunology since the time it was established back in the seventies[6].

CLINICAL USEFULNESS OF POLLEN COUNTS

Pollen counts are still highly useful for the clinician. They are essential for the identification of pollens causing pollinosis in each given township or geographic area[7]. They allow us to know with greater precision when the patients should begin their

Fig. 5. The Burkard Seven-Day Volumetric Spore-Trap®. It is based upon the same principle as the Hirst device, but allows uninterrupted sampling throughout seven days.

prophylactic treatment, and when they should stop it. They represent a considerable help in the planning of leisure and occupational travel of pollinosis patients, and they also provide help towards a better understanding of the variability in symptom severity over time and across geographic areas. In the latter context, continuous pollen count monitoring is essential because of the considerable interannual variations of one given pollen type[8]. As an example, the grass pollen concentrations in the city of Madrid may evidence up to 400% variation from one year to another, and these variations correlate significantly with those in antihistamine sales and in emergency care clinic attendance because of asthma in different seasons[9,10].

Pollen count monitoring allows us to detect increases of allergenic pollens in the atmosphere as a consequence of climatic/meteorological changes and of the increase and spread of allergenic plants (for instance, *Ambrosia* in Europe) or trees (e. g., *Cupressus, Platanus,* etc.) that are inductors of pollinosis. Pollen counts are essential in clinical studies of the efficacy of drugs and immunotherapy for seasonal rhinitis and/or asthma. Also, the pollen counts can help explain the greater or lesser prevalence of sensitisation to a given type of pollen in areas that are relatively close to each other[11,12].

PUBLICATION IN MASS INFORMATION MEDIA (ADVANTAGES AND DISADVANTAGES)

Even so, and despite their great usefulness for the clinician, it is at least curious that the most widespread use of pollen counts is the probably least effective one of them all: to provide daily information to the patients through the mass media (television, teletext, internet, printed daily press, radio). A recent study revealed that at least 49 million US citizens follow the pollen counts through television on a daily basis[13]. In theory, the aim of this information is to enable the patients to predict the expected severity of their pollinosis symptoms and thus be able to enhance their precautions regarding both avoidance and therapeutic measures "on that day". The pollen counts are usually presented by newsmen or by meteorologist who are in most cases unaware of the methodology used for the counts and, much more important, of the correct interpretation of such counts[13]. It is obvious that, for this information to be truly useful, the allergologists must teach the patients (and ideally also the mass media professionals who provide the information) the advantages but also the limitations of pollen counts, so as to avoid false expectations that may confuse rather than guide the patients in the control of their pollinosis symptoms.

DOSE - RESPONSE CURVES (LIMITATIONS)

Although, overall, a significant correlation may be found between the pollen counts and the mean of the rhinoconjunctivitis and asthma symptoms in selected groups of pollinosis patients who have noted their daily symptoms in specific diary cards, it is evident that if the study is individualised, patient-by-patient, that statistically significant dose - response curve will be missing in a variable number of cases[14-16].

One example: in linear correlation studies carried out in Madrid, a significant association between the severity of the rhinoconjunctivitis symptoms and the grass pollen counts was observed for only 56% of the patients, and between such symptoms and the *Olea* pollen counts in only 14%. Most pollinosis patients in Madrid are polysensitised to grasses and *Olea,* and it is quite probable that the former might have masked the possible correlation to be found to the latter. This correlation might also be further obscured by *Plantago,* which has the same pollination period as the grasses and *Olea* and sensitises 53% of the patients[16,17].

The range of severity of the pollinosis symptoms is enormously variable from one patient to another, so that what some of them consider to be a "high" count may be a "low" one for some others.

A further problem is that in the triggering of symptoms a number of pollen allergens may contribute that are not contained in the pollen grain themselves but are associated to particles less than 10 µm in size; the atmospheric concentrations of such particles may be at considerable variation to the pollen counts themselves[18-23].

Many other factors may increase the personal and individual exposure to pollens (use of automotion, open-air work, etc.) and/or to their allergens (e. g., lawn mowing)[24-26].

Turning to the methodology, a daily count is derived from the examination with a 40X objective of four longitudinal 48-mm sweeps of the glass slide (representing *ca.* 12% of the area actually impacted over one day); the numbers of pollens counted in those sweeps are then multiplied by a conversion factor (0.55), yielding the mean number of pollen grains in 1 m^3 of air. This means that there might have been times during the day in which the concentrations were much higher than those stated as the daily means; furthermore, the daily means provided to (and by) the mass media are actually –and logically– those of the previous day. As these mean counts are then transmitted to the public together with the weather report and the present temperature readings (within the last hour), this might induce error regarding the actual presence of pollens at the time the information is given.

The results of the pollen counts may also be influenced by the siting and height of the sampler spore-trap, and thus may not exactly represent the concentrations measured by another spore-trap sited nearby[27,28]. In this context, Ogden et al.[3] have carried out a number os studies that led to the development of guidelines regarding the siting of the samplers. Thus, the devices should be sited some 10 to 20 m (some 33 to 65 ft) above ground level, far from neighbouring tall buildings and other obstacles and also far from trees or other local pollen sources. Even when these guidelines are followed, some pollen sources may produce considerable variations in the counts in different sampler devices within the same city, as the pollen load in the atmosphere may actually be a not-at-all-homogeneous mixture, and particularly so when the source of pollen emission is relatively near the sampling device[27]. A greater homogeneity has thus been demonstrated for the grass pollen counts (the source of which is located outside the city) than for those of urban tree pollens such as *Platanus,* for which the emitting source is usually much closer to the sampler.

A further element that may influence the interpretation of pollen counts is the problem represented by the similarity under the light microscope of the pollens from different species, genera and even families of pollinating plants. The best example is that of the *Chenopodiaceae* and *Amaranthaceae* pollens; these are impossible to differentiate in optic microscopy, so that the counts for both families must be stated as a single pollen type (*"Chenopodio-Amaranthaceae"*). The problem becomes more complex when it is impossible to distinguish, within one pollen type, those from genera of widely varying allergenicity (for instance, it is quite impossible to differentiate the pollen grains from the scarcely-allergenic Urtica from those of the highly-allergenic *Parietaria*). Even though there are slight differences in the size of the pollen grains of these two genera, those differences are useless in practice because of the large intra-species variability range; once again, the counts must be expressed jointly as *"Urticaceae"*[30]. A further complication arises from the impossibility to differentiate species with considerable cross-reactivity. Typical examples of this problem may be the widespread use of *Plantago lanceolata* for diagnostic and immunotherapeutic purposes in Madrid instead of *Plantago lagopus,* which is more abundant and for which up to 21% more sensitisations are observed in the skin tests[17], or the case of *Phleum pratense,* a species that is practically nonexistent in the city of Madrid but is quite extensively used for immunotherapy in this city instead of *Trisetum paniceum,* which is the truly prevalent grass species but has only incomplete allergen identity to *Phleum*[17,31,32].

One other factor that must be borne in mind in the interpretation of pollen counts is the fact that the response threshold, both nasal and bronchial, gradually decreases throughout the season *(priming).* The first investigator to report this particular effect was Connell[33], who found that in nasal challenge tests performed in successive days with *Ambrosia*

pollen the minimum number of pollen grains required for the induction of symptoms decreased gradually. He also observed that the pollen grain concentration required for triggering symptoms was significantly lower in mid-season and at the end of the season than at its beginning. This priming effect has also been reported with *Betula* pollen; it has been observed that 90% of the patients with clinical *Betula* sensitisation develop symptoms with pollen counts higher than 80 grains/m^3 at the beginning of the season, decreasing to 30 grains/m^3 at its end. Furthermore, in patients with *Betula* allergy the reactivation threshold may be further diminished by the presence in the atmosphere, a few weeks earlier, of other pollens with which *Betula* shares antigens (*Alnus* and *Corylus*). Conversely, *Betula* also lowers the reactivation threshold in patients sensitised to the grasses that pollinate at almost the same time in Scandinavia[8]. It is evident that the symptoms are a reflection of the exposure to a number of allergens and that the response threshold is influenced by the interaction between them; this explains the difficulties encountered in establishing precise reactivation thresholds. Even so, it has been established that grass pollen concentrations ranging from 10 to 50 grains/m^3 air[16,34-36], and *Olea* pollen concentrations ranging from 153 to 400 grains/m^3 air are able to reactivate most clinically sensitised patients[37-38].

A number of genetic factors of the plants and trees may have effects on the allergenicity of their pollens. For instance, the mean allergen levels in *Betula* pollen may be inherited; as this particular tree tends to disperse its seeds locally, it is quite possible to find regional differences in the pollen allergenicity among groups of trees of the same species[39].

The degree of environmental pollution and the variations in temperature also have an effect on the trees and may induce an increase in the allergen contents of their pollens. Differences in pollen allergenicity have been observed between groups of trees of the same species that, although growing in relative (geographic) proximity, do actually grow in areas with different pollution levels (city / open fields) or with different temperatures (valley / mountain)[39].

Environmental pollution may also affect pollen allergenicity through a direct effect on the pollen grain itself. It has been demonstrated that particulate matter derived from the combustion of Diesel fuel coats the pollen grains collected near highways and motorways[40]. This particulate matter may have an adjuvant effect, as demonstrated by Miyamoto and coworkers at the University of Tokyo over 15 years ago: in experimental studies in mice they observed that the IgE response to allergens of Japanese cedar *(Cryptomeria)* pollen increased significantly when the pollen grains were mixed with particulate matter from Diesel fuel combustion[41]. A number of epidemiologic studies have shown that the prevalence of hay fever in the urban environment is twice that in the rural one, even though the pollen concentrations are higher in the latter[42,43]. Furthermore, even in the rural environment differences occur, as reported by Ishizaki et al.[42], who observed that the prevalence of hay fever due to *Cryptomeria* sensitisation among Japanese peasants residing close to highways was almost threefold that in those living farther away (13% vs. 5%).

There are also differences within the urban environment, as pointed out by Luczynska. This investigator observed that the prevalence of grass pollen sensitisation among 10-11-year-old schoolchildren in the highly contaminated urban centre of London was 34%, *versus* 20% among schoolchildren of the same age living in a much-less-contaminated residential area in Southern London. More recently, Díaz Sánchez et al., in a study on 13 patients diagnosed of *Ambrosia* sensitisation, demonstrated that the increase in the specific IgE levels in the nasal secretions four days after a nasal challenge test with *Amb a*1 (the major allergen of *Ambrosia*) mixed with particulate matter from Diesel fuel combustion were 16-fold higher than those elicited by a challenge test with Amb a1 without Diesel particles.

Particulate matter from Diesel exhausts has been shown to be able to absorb airborne allergens (for instance, *Lol p*1), to act as atmospheric carriers for those allergens and to prolong allergen retention. Upon being phagocyted by the macrophages and other cells of the respiratory tract mucosa they induce a considerable increase in the production of important proinflammatory cytokines and in the accumulation of eosinophils in the airway mucosa.

This particulate matter may also exert a proinflammatory effect in non-allergic patients and exacerbate both intrinsic and extrinsic bronchial asthma. It is also able to decrease mucociliary clearance and

to increase the permeability of airways mucosal cells to allergens[43,44].

Considering all these data together it is logical to suspect that the reactivation threshold for pollens is also influenced by the pollution / contamination levels and particularly to the levels of particulate matter from Diesel exhausts, a further highly important variable to consider in the interpretation of pollen counts.

CONCLUSIONS

Pollen counts are an essential tool for the Allergologist in research, in the diagnosis and in the therapeutic management of pollinosis patients. Their usefulness as daily information for the patients during the pollenation season is rather less and is largely dependent upon the patients correctly interpreting those counts. This correct interpretation should be taught by the physicians.

REFERENCES

1. Blackely CH. Experimental Researches on the Nature and Causes of Catarrhus Aestivus. Londrés; Bailliere, Tindal & Cox, 1873.

2. Durham OC. The volumetric incidence of airborne allergens. IV. A proposed standard method of gravity sampling, counting and volumetric interpolation of results. J Allergy 1946;17:79-86.

3. Ogden EC, Raynor GS, Hayes JV. Manual for sampling airborne pollen. Nueva York; Hafenr press, 1974.

4. Hirst JM. An automatic volumetric spore trap. Ann Appl Biol 1952; 39:257-265.

5. Bush RK. Aerobiology of pollen and fungal allergens. J Allergy Clin Immunol 1989;84:1120-1124.

6. Subiza E, Subiza J, Jerez M. Palinología. En: Tratado de Alergología e Inmunología Clínica. Vol IV. Madrid: Luzán, 1986; 211-254.

7. Solomon WR, Mathews KP. Aerobiology and inhalant allergens. En: Middleton E, Reed Ch, Ellis EF, Adkinson NF, Yunginger JW, ed. Allergy: principles and practice, Vol 1, 3rd ed. San Luis; CV Mosby, 1988;312-313.

8. D'Amato G, Spieksma FThM, Bonini S. (eds). Allergenic pollen and pollinosis in Europe. Londrés: Blackwell Scientific Publications, 1991.

9. Subiza J, Jerez M, Gavilán MJ, Varela S, Rodríguez R, Narganes MJ, et al. ¿Cuáles son los pólenes que producen polinosis epidémica en el medio urbano de Madrid? Rev Esp Alergol Inmunol Clin 1998; 13:107-109.

10. Red de médicos centinela. Bol Epidemiol Comun Madrid. Mayo-Junio 2000 (supl.); 6:37.

11. Zwick H, Popp W, Jäger S, Wagner C, Reiser K, Mora KF. Pollen sensitization and allergy in children depend on the pollen load. Allergy 1991;46:362-366.

12. Subiza J, Feo Brito F, Pola J, Moral A, Fernández J, Jerez M, et al. Pólenes alergénicos y polinosis en 12 ciudades españolas. Rev Esp Alergol Inmunol Clin 1998; 13:45-48.

13. Frenz DA, Goswami AM, Murray LW, Lince NL. A survey of television meteorologist about their sources for and understanding of pollen counts. Ann Allergy 1998;81:439-442.

14. Subiza J, Cabrera M, Valdivieso R, Subiza JL, Jerez M, Jiménez JA, et al. Seasonal asthma caused by airborne *Platanus* pollen. Clin Exp Allergy 1994;24:1123-1129.

15. Varela S, Subiza J, Subiza JL, Rodríguez R, García B, Jerez M, et al. Platanus pollen an important unrecognized cause of pollinosis. J Allergy Clin Immunol 1997;100:748-754.

16. Muñoz M, Subiza J, Gavilán MJ, Barjau C. ¿Es posible establecer una curva dosis-respuesta entre los recuentos de pólenes de gramíneas y síntomas de rinitis? Rev Esp Alergol Inmunol Clin 2000; 15:17.

17. Subiza J, Jerez M, Jiménez JA, Marganes MJ, Cabrera M, Varela S, et al. Allergenic Pollen and pollinosis in Madrid. J Allergy Clin Immunol 1995;96:15-23.

18. Solomon WR, Burge HA, Muilenberg ML. Allergen carriage by atmospheric aerosol. I. Ragweed pollen determinants in smaller micronic fractions. J Allergy Clin Immunol 1983;72:443-447.

19. Agarwal MK, Swanson MC, Reed CE, Yunginger JW. Airbone ragweed allerllens: association with various particle sizes and short ragweed plant parts. J Allergy Clin Immunol 1984;74:687-693.

20. Fernández-Caldas E, Swanson MC, Pravda J, Welsh P, Yunginger JW, Reed CE. Immunochemical demonstration of red oak pollen aeroallergens outside the oak pollination season. Grana 1889; 28: 205-209.

21. Knox RB. Grass pollen, thunderstorms and asthma. Clin Exp Allergy 1993;23:354-359.

22. Spieksma FThM, Nikkels AH, Kijkman JG. Seasonal appearance of grass pollen allergen in natural pauci-micronic aerosol of various size fractions. Relationship with airborne grass pollen concentration. Clin Exp Allergy 1995;25:234-239.

23. Cabrera M, Martínez Cócera, Boluda L, Tejada J, Subiza JL, Subiza J, et al. Presencia de aeroalergenos de gramíneas en la atmósfera de Madrid. Rev Esp Alergol Inmunol Clin 2000;15:16.

24. Muilenberg ML, Skellenger WS, Burge HA, Solomon WR. Particle penetration into the automotive interior. I. Influence of vehicle speed and ventilatory mode. J Allergy Clin Immunol 1991;87:581-585.

25. D'Amato G, Russo M, Liccardi G, Saggese M, Gentili M, Mistrello G, et al. Comparison between outdoor and indoor airborne allergenic activity. Ann Allergy Asthma Immunol 1996;77:147-152.

26. Subiza J, Subiza JL, Hinojosa M, Varela S, Cabrera M, Marco F. Occupational asthma caused by grass juice. J Allergy Clin Immunol 1995;96:693-695.

27. Frenz DA. Interpreting atmospheric pollen counts for use in clinical allergy: spatial variability. Ann Allergy Asthma Immunol 2000; 84:481-491.

28. Hart ML, Wenworth JE, Bayley JP. The effect of trap height and wether variables on recorded pollen concentration at Leicester. Grana 1994;33:100-103.

29. Bryant RH, Emberlin JC, Norris-Hill J. Vertical variation in pollen abundance in North-Central London. Aerobiologia 1989;5:123-137.

30. Jelks ML. Interpretation of pollen counts. Ann Allergy 1991; 67:1-2.

31. Subiza E, Subiza J, Jerez M. Árboles, hierbas y plantas de interés alergológico en España. En: Tratado de Alergología e Inmunología Clínica, Tomo IV. Madrid: Ed. Luzan, 1986; 257-365.

32. Montero MT, López C, Jiménez JA, Subiza J. Characterization of allergens from *Trisetum paniceum* pollen: an important aeroallergen in Mediterranean continental climatic areas. Clin Exp Allergy 1997; 12:1442-1448.

33. Connell JT. Quantitative intranasal pollen challenges. III. The priming effect in allergic rhinitis. J Allergy 1969;43:33-44.

34. Davies RR, Smith IP. Forecasting the start and severity of the hay fever season. Clin Allergy 1973;3:263-267.

35. Ickovic MR. The French aerobiological monitoring network: Two years of clinical experience (1986-1987). Aerobiologia 1988;4:12-5.

36. Antépara I, Fernández JC, Gamboa P, et al. Pollen allergy in the Bilbao area (European Atlantic seaboard climate): pollination forecasting methods. Clin Exp Allergy 1995;25:133-140.

37. Florido JF, Delgado PG, de San Pedro BS, Quiralte J, de Saavedra JM, Peralta V, et al. High levels of Olea europaea pollen and relation with clinical findings. Int Arch Allergy Immunol 1999; 119(2):133-137.

38. Feo Brito F, Cárdaba B, Lahoz C, Subiza J, Jerez M, López G. Oleaceae. En: Atlas de Aerobiología y polinosis. Madrid: Lab. Shering-Plough SA, 2000.

39. Ahlholm JU, Helander ML, Savolainen J. Genetic and environmental factors affecting the llergenicity of birch (Betula pubescens ssp. czerepanovii [Orl.] Hamet-ahti) pollen. Clin Exp Allergy 1998; 28:1384-1388.

40. Behrendt H, Becker WM, Friedrisch KH, et al. Interaction between aeroallergens and airborne particulate matter. Int Arch Allergy Immunol 1992;99:425-428.

41. Muranaka M, Suzuki S, Koizumi K, Takafuji S, Miyamoto T, Ikemori R, et al. Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. J Allergy Clin Immunol 1986; 77(4):616-623.

42. Ishizaki T, Koizumi K, Ikemori R, Ishiyama Y, Kushibiki E. Studies of prevalence of Japanese cedar pollinosis among the residents in a densely cultivated area. Ann Allergy 1987;58:265-270.

43. D'Amato G. Urban air pollution and plant-derived respiratory allergy. Clin Exp Allergy 2000;30:628-636.

44. Knox RB, Suphioglu C, Taylor P, Desai R, Watson HC, Peng JL, et al. Major grass pollen allergen Lol p 1 binds to diesel exhaust particles: implications for asthma and air pollution. Clin Exp Allergy 1997;27:246-251.