Pollinosis due to Australian pine (Casuarina): an aerobiologic and clinical study in southern Spain


An aerobiologic and clinical survey was conducted in Málaga, southern Spain, in order to determine fluctuations of Australian pine (Casuarina) pollen in the atmosphere of the city, and the prevalence of sensitivity in a nonatopic population. The aerobiologic survey, using a Burkard spore trap, was conducted from January 1991 to December 1994, and sensitization was ascertained by the skin prick test. The pollen season is relatively short and the pollen dispersion period occurs during October and November, mainly during the last 3 weeks of October. Diurnal patterns showed that the highest concentrations of pollen occur between 12 a.m. and 2 p.m., the most influential variables in its dispersion being temperature, sunshine, and rainfall. The prevalence of sensitization to Casuarina pollen was determined by skin prick test (SPT) in a nonatopic population of 210 patients with a previous history of autumn rhinitis, asthma, or rhinitis asthma. Six subjects showed a positive reaction to the pollen extracts, and the presence of specific IgE was demonstrated by the conventional radioallergosorbent test (RAST ≥2) in five of these patients with positive SPT.

The genus Casuarina comprises about 60 species that are mainly native to Australia, although some are found in southern Asia. Some of these species are cultivated as ornamental plants in tropical and subtropical regions and are commonly known as “Australian pine” because of their resemblance to trees of the genus Pinus.

They are wind-pollinated trees that produce a very large number of pollen grains whose allergenicity has been demonstrated (1-3). Australian pine pollen allergenicity has previously been reported by Zivit in 1942 (3), who described three patients with hay fever and/or asthma coinciding with the pollination of Australian pine trees. The three patients had positive intradermal tests, and specific “reagins” were demonstrated in two of the patients by a passive transfer test.

In 1950, Guttmann (4), suspected the possible allergenicity of Casuarina pollen but reported no clinical data. Thirty-seven years later, Bucholtz et al. (1) reported that 14/61 volunteers had positive skin prick test (SPT) for Australian pine pollen, and 11 of these subjects had a positive RAST score of 1+ or greater. In 1989, Jelks (5) reported that commercial pollen extracts gave about 29% reactivity in atopic subjects in Florida, but she did not describe the method used. More recently, García Ramos et al. (2) found that of 501 high-school students in Tenerife (Canary Islands, Spain), 11 (2.1%) had positive SPT.

Although the incidence of Casuarina allergy in the European population as a whole has not been determined, many studies have mentioned the airborne pollen of Casuarina, not only in southern Spain (6-8), but also in several other Mediterranean cities (9-13), and it has also been described in Florida (5, 14), Havana (15), Caracas (16), and Buenos Aires (17).

The most common species of Australian pine cultivated and occasionally naturalized in Málaga (southern Spain) is Casuarina cunninghamiana (Fig. 1), which is frequently found in gardens and hedges because it is resistant to the dry climate. In this coastal city (latitude 36°37' N,
longitude 4°19' W) with its typically Mediterranean climate, the flowering season of *C. cunninghamiana* is October and November, but mainly October. This has already been reported in the aerobiologic survey carried out in Málaga in 1982–5 (18).

The pollen grains are trizonoporate, triangular in polar view, with convex sides and the pores situated in the angles. This pollen type resembles those of *Corylus* (hazel), *Betula* (birch), and *Myrica* (wax myrtle), but it is larger.

Of patients aged 14–56 years who attend our clinic for the first time and are subjected to SPT because of suspected rhinitis and/or asthma, 2–3% (2.6%) show a positive reaction to this pollen, although most also test positive to other pollens (especially Gramineae and olive) and/or dust mites. To detect the possible existence of monosensitive subjects, we examined a group of 210 patients with respiratory problems (which were particularly acute) in autumn, coinciding with the pollination period of the species in question, as a first step toward establishing a possible relationship with *Casuarina* pollen. This work aimed to determine the atmospheric fluctuations and the existence of patients monosensitized to *Casuarina* pollen in a selected population by SPT.

**Material and methods**

**Aerobiologic sampling**

The sampling was carried out with the aid of a Burkard spore trap located on the roof of the Faculty of Medicine of the University of Málaga about 15 m above ground level. This faculty is situated 1 km west of the city center, in an open space with no surrounding buildings to obstruct the free circulation of air. The pollen data are expressed in grains per cubic meter (daily average) and were obtained by counting, hour by hour, four lengths of the slide with a ×40 objective (0.45-mm field), by the method of the Spanish Aerobiology Network (REA) (19).

**Statistical data**

To determine the daily variation patterns, we considered only dry days, with no rainfall, when the number of pollen grains was equal to or more than the daily average for the 95% of the total annual pollen count, from the moment the cumulative sum reached a value of 2.5%. The correlations between pollen concentrations and meteorologic variables were obtained by the Pearson product-moment correlation. The following meteorologic variables were used in the study: mean temperature, sunshine, rainfall, relative humidity, and wind velocity. For temperature, sunshine, and rainfall, we used the cumulative values from 22 June (summer solstice), since *C. cunninghamiana* blooms in autumn. The pollen data were converted to logarithmic values in order to obtain normal distribution curves. Meteorologic data were provided by the Meteorologic Institute of Málaga.

**Subjects**

The study comprised 210 volunteers (102 men and 108 women), aged 18–44 years (mean 26.32) from the outpatient services of the Allergy and Immunology Service of the Carlos Haya Hospital.
in Málaga. All patients resided in the Málaga metropolitan area and had a previous history of rhinitis (87.6%), asthma (8.1%), or rhinitis asthma (4.2%), principally from September to January, but not during the rest of the year.

All the patients were previously tested and had negative SPT against a battery of the most common pollens, mites, fungal spores, and extracts of animal dander used in southern Spain (pollen: Olea europaea, Platanus orientalis, Fraxinus excelsior, Quercus ilex, Lolium perenne, Cynodon dactylon, Parietaria judaica, Chenopodium album, Plantago lanceolata, and Artemisia vulgaris; mites: Dermatophagoides pteronyssinus and D. farinae; fungal spores: Alternaria tenuis, Aspergillus fumigatus, and Cladosporium herbarum; animal dander: cat and dog).

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**Pollen extract**

Australian pine pollen extracts were prepared at a 10% (w/v) ratio in 50 mM ammonium bicarbonate by magnetic stirring for 4 h at 4–8°C. After centrifugation (39,000 g/min), the preparation was extensively dialyzed against distilled water and filtered through a 0.22-µm-pore-size membrane. Sterile glycerin and NaCl were added to a final concentration of 50% (v/v) and 0.9% (w/v), respectively. These extracts were supplied by Alergia e Inmunología Abello (Madrid, Spain).

**Skin test**

SPT were performed with commercially available glycerinated extracts (Alergia e Inmunología Abello) on the 210 patients and 20 nonatopic subjects (without symptoms and with negative SPT) to exclude false-positive results. Histamine chlorohydrate (10 mg/ml) was used as a positive control and sterile water solution of NaCl as a negative control. SPT reactions were regarded as positive if the wheal size was at least 3 mm in diameter or ≥50% of the histamine reactions. A reaction half the histamine reaction size was recorded as positive (2+). The reactions to *Casuarina* extracts were read 15 min after SPT, and SPT were conducted during the nonflowering season of *Casuarina*, from 11 a.m. until 13 p.m., on the anterior aspect of the forearm (20), always by the same person and method. Following the recommendations of the EAACI Immunotherapy Subcommittee (21), none of the subjects received any medication before or at the time of testing.

The accuracy of the technique was verified by analyzing the results obtained in 10 healthy patients submitted to quadruple histamine prick tests (coefficient variation 23.48%).

**Specific IgE determination**

Specific IgE determinations were performed with commercial RAST reagents (Phadebas® Pharmacia). For *Casuarina*-specific IgE determinations, disks coupled to pollen extracts were prepared according to Ceska et al. (22). RAST classes equal to or greater than 2 were considered positive.

Specific IgE levels to *D. pteronyssinus* (the most important allergen in the pathogenesis of rhinitis and asthma during autumn and winter in the study area) were determined.

**Results**

**Aerobiologic survey**

The dispersion period for *Casuarina* pollen in Málaga occurs during October and November, mainly from the second to the last week of October (Fig. 2), although the total annual pollen counts show significant fluctuations from year to year. Thus, during 1991 and 1992, the counts did not exceed 300 grains/m³ (daily average sum), while, in 1993 and 1994, they were 786 and 717, respectively.

The length of the main pollen season (95% of total annual) ranged from 21 days in 1994 to 31 days in 1993, although the peak days always occurred during October. These peaks had different values for the 4 years that were studied, and were significantly higher in the last 2 years of the study: 44 grains/m³ in 1991 (25 October), 25 in 1992 (27 October), 166 in 1993 (22 October), and 221 in 1994 (10 October). During the 4-year study, *Casuarina* accounted for 0.8–2.5% of the total annual pollen counts recorded in Málaga but 41.1–78.6% of the total for October (Table 1).

For the daily variation (Fig. 3), we observed a very similar pattern during the 4 years studied, the maximum concentrations (about 44% of the daily total) occurring between 12 a.m. and 2 p.m. (local time, –1 h GMT).

During 1991, 1992, and 1993, the correlations between pollen levels and temperature, sunshine, and rainfall showed positive values during October but negative values in November. Both positive and negative values were always highly significant (P < 0.001) except for rainfall in November 1991. However, no significant correlations were found for the same variables for 1994. For relative humidity and wind velocity, the correlations obtained showed no very clear trends (Table 2).

**Clinical study**

Six of the 210 patients (2.85%), four women and two men, had a positive skin reaction to the
Fig. 2. Pollen counts and rainfall in Málaga during main *Casuarina* pollen season.

Table 1. Total pollen counts of Australian pine pollen (grains/m$^3$), duration of main pollen season, peak days, and percentages compared with total annual and October pollen counts

<table>
<thead>
<tr>
<th></th>
<th>Total annual (grains/m$^3$)</th>
<th>Main pollen season (95%)</th>
<th>Total days</th>
<th>Peak day</th>
<th>Highest daily count (grains/m$^3$)</th>
<th>% annual</th>
<th>% out of October</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>294</td>
<td>5 Oct to 19 Nov</td>
<td>25</td>
<td>20 Oct</td>
<td>44</td>
<td>50</td>
<td>46.9</td>
</tr>
<tr>
<td>1992</td>
<td>277</td>
<td>7 Oct to 13 Nov</td>
<td>28</td>
<td>27 Oct</td>
<td>25</td>
<td>1.2</td>
<td>41.1</td>
</tr>
<tr>
<td>1993</td>
<td>786</td>
<td>15 Oct to 14 Nov</td>
<td>31</td>
<td>22 Oct</td>
<td>166</td>
<td>2.5</td>
<td>78.6</td>
</tr>
<tr>
<td>1994</td>
<td>717</td>
<td>10 Oct to 30 Nov</td>
<td>21</td>
<td>10 Oct</td>
<td>221</td>
<td>2.5</td>
<td>77.4</td>
</tr>
</tbody>
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*Casuarina* pollen extracts, while the skin reaction was negative to the pollen extracts of the remaining allergens tested. All had rhinitis and one (woman) had asthma and rhinitis. Five of these six cases
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(83%) had positive RAST; only one was negative (Table 3).

RAST for Dermatophagoides was always negative, and SPT of the 20 nonatopic patients was also negative.

Discussion

Aerobiologic survey

In Málaga, Australian pine pollen shows very stable seasonal trends like those observed in nearby cities such as Córdoba (7) and Seville (8), where this pollen type is detected during autumn, mainly during October and November, probably because this genus is represented by the same species: C. cunninghamiana. Nevertheless, in other countries, where different species, such as C. equisetifolia, that bloom in spring are cultivated, peaks in pollen levels have been detected in the atmosphere during March (5, 14).

As shown in Fig. 2, the start of flowering appears to be determined by the first autumn rainfalls, and the maximum peak concentrations are detected during the sunny days which follow. An evident increase in the pollen counts recorded was observed throughout the 4-year study, probably because Australian pine has been extensively planted during the last few years as a windbreak along roadsides and in dry places because of its resistance to drought and rapid growth.

Although the annual pollen counts for Casuarina do not exceed 2.5% of the total annual count, this is the highest percentage reported, to our knowledge, in the literature. Furthermore, during the last 2 years of study, this pollen type accounted for almost 80% of the total pollen count for October (Table 1). Therefore, we believe that Australian pine pollen should be taken into account as a possible cause of autumn pollinosis in southern Spain.

The diurnal trends of Casuarina also showed a very stable pattern throughout the study, with slight differences from year to year and very sharp peaks around midday (Fig. 3). The daily variation of this species has been previously studied by Galán et al. (23) in Córdoba, where it showed a more variable behavior.

During 1991, 1992, and 1993, the correlations obtained showed that cumulative mean temperature, sunshine, and rainfall are the most influential variables in the pollen dispersion of this species (Table 2), but these correlations were positive only during October. The same variables become negative and sometimes even independent during November when flowering is terminating and atmospheric pollen decreases. There are no significant values for rainfall during November 1991 simply because there was no rain. In 1994, Australian pine pollen showed atypical behavior, the main pollen season lasting only a few days, and this might be the reason we found no significant correlations.
with any of the atmospheric variables. We used the cumulative values from after the summer solstice because temperature and sunshine decrease during autumn and rainfall has a delaying effect on pollen production.

Finally, the correlations showed no clear trends for relative humidity and wind velocity. It might be expected that an increase in wind velocity would facilitate atmospheric pollen dispersion, but it is possible that the results were influenced by wind direction, which is very variable in Málaga. However, relative humidity is always high since this is a seaside city.

Clinical research

In the present study, for the first time in Europe, we describe the prevalence of positive skin tests and the existence of specific IgE to Australian pine pollen. We found that this prevalence, in a very selected population, is low (2.85%). However, it is important to emphasize that the six subjects with positive SPT were monosensitive and there was a very good correlation between SPT and RAST. Furthermore, if positivity is defined as a RAST score of 1+ or greater, as in the study of Bucholtz et al. (1), the correlation would be 100%. Furthermore, the patients who are sensitized to mites (principally *D. pteronyssinus* and *D. farinae*) in our area suffer an increase in sensitization in October. Given the characteristics of the population studied, the sensitization to mites should be discounted. However, both SPT and RAST were negative to these allergens in the 210 patients studied.

Pollen grains from trees of the Fagales order (alder, birch, hazel, and oak) are the main allergen source in the temperate zone of the Northern Hemisphere during spring, and patients sensitized to pollen of this type often exhibit reactions to pollen of this type. In our area, we describe the prevalence of positive skin tests and the existence of specific IgE to Australian pine pollen. We found that this prevalence, in a very selected population, is low (2.85%). However, it is important to emphasize that the six subjects with positive SPT were monosensitive and there was a very good correlation between SPT and RAST. Furthermore, if positivity is defined as a RAST score of 1+ or greater, as in the study of Bucholtz et al. (1), the correlation would be 100%. Furthermore, the patients who are sensitized to mites (principally *D. pteronyssinus* and *D. farinae*) in our area suffer an increase in sensitization in October. Given the characteristics of the population studied, the sensitization to mites should be discounted. However, both SPT and RAST were negative to these allergens in the 210 patients studied.

Pollen grains from trees of the Fagales order (alder, birch, hazel, and oak) are the main allergen source in the temperate zone of the Northern Hemisphere during spring, and patients sensitized to pollen of this type often exhibit reactions to another pollen of the same order (24). The amino acid sequence of the allergens of this species is highly correlated, and the NH₂-terminal amino acid sequences of the major allergens exhibit approximately 80–90% of sequence identity (24). *Casuaria* is a genus closely allied to families such as Betulaceae, Fagaceae, and Myricaceae, and its pollen grains closely resemble those of other genera of these families, such as *Betula* (birch), *Corylus* (hazel), and *Myrica* (wax myrtle or bayberry), for which they are easily mistaken. Bucholtz et al. (1) suggested that *Casuaria* may cross-react with *Myrica*, *Morus* (mulberry), and *Quercus* (oak). Spieksma (25) indicated a certain degree of cross-reactivity among the Betulaceae genera (*Alnus*, *Betula*, and *Corylus*). Moreover, some studies conducted by Pauli et al. (26) and van Dalen & Voorhorst (27) have suggested that there is a high degree of allergenic similarity between Betulaceae and Fagaceae.

We believe that a similarity of allergenic properties within the Betulaceae, Fagaceae, and Casuarinaceae families is not unlikely, and possible cross-reactivity between the *Casuaria* and the above-mentioned genera should be investigated. Australian pine is a very common tree in many cities of the Old World and an abundant source of windborne pollen. Thus, we believe, it must be considered an aeroallergen and a probable cause of allergic diseases. The presence of this pollen type in the atmosphere of Málaga shows very regular patterns; therefore, its extracts have been included in the standard SPT for patients with rhinitis and/or asthma in order to determine the prevalence of allergy to it.

Acknowledgments

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References

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