ARQUIVOS DE ASMA, ALERGIA E IMUNOLOGIA

Original Article

Seasonal changes in *Poaceae* pollen counts in Curitiba, south of Brazil

Mudanças na sazonalidade de polens de Poaceae *em Curitiba*

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ABSTRACT

Background: Allergic diseases affect 10% to 30% of the world population, with pollen as a major trigger. Pollinosis results from sensitization to pollen and is the seasonal form of allergic rhinitis and/or immunoglobulin E (IgE)-mediated allergic asthma. The *Poaceae* family is distributed worldwide and has the largest number of plant genera contributing to pollinosis, as they release large amounts of pollen into the atmosphere. **Objective:** To quantify pollen grains from the *Poaceae* family in the atmosphere of Curitiba, compare the pollen distribution curve with data from the 1980s and 1990s, and classify the daily concentration of grass pollen according to the *National Allergy Bureau* (NAB). Method: A Hirst-type volumetric sampler was placed at approximately 25 meters from the ground. **Results:** The peak of daily total pollen concentration occurred in early August, corresponding to 302 grains/m^3. August also had 8 of the highest daily total pollen concentrations, 7 of which were greater than 200 grains/m^3. *Poaceae* pollen was found throughout the year, with the highest concentration peak of 27 grains/m^3 in August and September. In the 1980s and 1990s, the pollen peaks occurred in November and the pollen season occurred between October and April. In 2018, however, the pollen season started earlier, in August, and the pollen peaks occurred in August and September. **Conclusion:** This study shows a change in the grass pollen season. Although the 2 peaks of *Poaceae* pollen dispersion have repeated over the years, grass pollen is currently observed in other months of the year. Patients with pollen allergy may experience symptoms from allergen exposure outside the previously established grass pollen seasons.

Keywords: Pollen, seasonal allergic rhinitis, allergic conjunctivitis.

RESUMO

Introdução: Doenças alérgicas afetam de 10 a 30% da população mundial, e polens são frequentes desencadeantes. A polinose é doença decorrente da sensibilização ao pólen e é a forma sazonal da rinite alérgica e/ou asma mediada pela imunoglobulina E (IgE). A família *Poaceae* tem o maior número de gêneros de plantas que contribuem para a polinose, pois liberam alta quantidade de pólen na atmosfera e são largamente distribuídas. **Objetivo:** O presente trabalho quantificou a concentração de polens da família *Poaceae* na atmosfera de Curitiba e comparou a curva de distribuição de polens com os dados das décadas de 1980 e 90. Também classificou a concentração diária de pólen de gramíneas segundo a *National Allergy Bureau* (NAB). **Método:** O equipamento de amostragem foi o captador volumétrico Hirst, instalado a uma altura de aproximadamente 25 metros. **Resultados:** O pico de concentração diária de pólen total ocorreu no começo do mês de agosto, correspondendo a 302 grãos/m^3. O mês de agosto também concentrou oito dos maiores picos diários de pólen total, sendo sete deles superiores a 200 grãos/m^3. Foi encontrado pólen *Poaceae* ao longo de todo o ano e o maior pico de concentração foi de 27 grãos/m^3 em agosto e setembro. Nas décadas de 80 e 90, os picos de polens foram no mês de novembro e período de polinização entre outubro e abril. Isso não foi observado no ano de 2018, uma vez que a época de polinização das gramíneas se adiantou, com início em agosto, e o pico de concentração foi em de agosto e setembro. **Conclusão:** Este estudo mostra que houve mudança na estação polínica. Os dois picos de dispersão de polens de *Poaceae* se repetem ao longo dos anos, mas têm sido encontrados em outros meses. Pacientes com alergia a polens podem ter sintomas por exposição fora das estações determinadas anteriormente.

Descritores: Pólen, rinite alérgica sazonal, conjuntivite alérgica.

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Introduction

Allergic rhinitis (AR) is an inflammatory reaction of the nasal mucosa characterized by sneezing, nasal itching, rhinorrhea, and nasal congestion in the absence of a cold.[1] Although sometimes mistakenly considered a trivial condition, symptoms can significantly affect one's quality of life and are associated with conditions such as fatigue, headache, cognitive problems, and sleep disturbances, affecting school and work performance.[2] Allergic rhinitis (AR) and allergic conjunctivitis are currently estimated to affect up to 40% of the world's population.[3,4] AR-related ophthalmic symptoms occur in 30-70% of patients and are more commonly triggered by indoor allergens.[5]

Pollen-induced rhinoconjunctivitis represents the most prevalent allergic disease, which is mediated by IgE antibodies and results from the interaction of chemical mediators, cytokines, and adhesion molecules with different cell types, such as endothelial cells, mast cells, lymphocytes, eosinophils, and basophils, among others. The consequence is allergic inflammation and nonspecific hyper-reactivity.[6]

Pollen is the most common airborne allergen and is a frequent trigger of allergic diseases in humans. Pollinosis is the pollen sensitization disease and is considered the acute seasonal form of allergic rhinoconjunctivitis and/or bronchial asthma mediated by IgE antibodies that recurs with the same periodicity.[7,8]

Tests to demonstrate IgE sensitization on the skin in serum or by mucosal provocation and pollen dispersal in the atmosphere are ways to demonstrate the cause of seasonal allergic symptoms. In Brazil, grasses are the main agent of pollinosis and of relatively recent identification.[5,8]

Among the non-native grass species that were introduced to Brazil by European immigrants is ryegrass (*Lolium multiflorum*), a species that has adapted very well to the Southern Region and can therefore be found growing rampantly across the cities of Brazil.[9] According to skin tests performed in pollinosis patients with extracts of different grass species, *Lolium multiflorum* was the species that caused the most allergic reactions; therefore, it is considered the main grass species causing pollinosis.[9-12]

The *Poaceae* family, more commonly called grasses, comprises 668 genera and approximately 10,000 species. In Brazil, about 1,500 species are recorded in the *Poaceae* family.[13]

Since the grass family is large, it was divided into subfamilies and tribes. The subfamilies that comprise 90% of the grass species and 95% of the immunologically relevant species are the subfamilies *Chloridoideae*, *Pooideae*, and *Panicoideae*.[14]

Despite the extensive distribution of grasses in the city of Curitiba, other anemophilous species have a large participation in the pollen concentration in the city's atmosphere along the year, a fact also observed in the municipality of Caxias do Sul, where *Poaceae* corresponded to 12% of the total pollen (TP).[15]

Studies in other countries have shown a wide range in the percentage of grass pollen. In Montevideo, Uruguay, *Poaceae* contributed 47% to 2013-2014 total pollen;[16] in San Carlos de Bariloche, Argentina, grasses contributed on average 6% to TP;[17] and in the city of Porto, Portugal, it reached 8% of total TP.[18]

Historically pollen sampling was done by the gravimetric method using the Durham sampler, with pollens recorded in an area of 1 cm^2. From this count, in 1981 and 1982 it was possible to observe two annual peaks of grass pollen concentration in Curitiba, the highest occurring in the second and third week of November and reaching 117 grains/cm^2, and a lower concentration peak (48 grains/cm^2) in March.[8] The most used method today is the volumetric method, in which pollen counts are expressed in number of grains per m^3 of air.[14]

The prevalence of pollinosis has increased in subtropical regions with well-defined seasons.[18-21] Deforestation associated with the climate of the southern states of Brazil and the introduction of non-native grass species have probably enabled the spread of *Poaceae* grasses in these regions.

Meteorological factors positively or negatively affect plant development, flowering, and pollen concentration in the atmosphere. Relative humidity and temperature influence pollen production and release, as they affect the formation and opening of the anthers for pollen release. Wind speed favors the release of pollen from anemophilous plants but dilutes the pollen concentration in the atmosphere. Finally, precipitation positively influences the concentration of pollen in the atmosphere if it occurs during plant growth since it generally increases pollen production in the plant. However, if precipitation occurs when pollen has already been released into the air, the influence is negative by washing out the biogenic particles.[21]

Air pollution and global warming stimulate plants to develop more, with higher pollen production and

higher allergen content; pollination occurs earlier and is longer and more intense. As a consequence, there will be more pollen allergy sufferers, new sensitizations, more intense symptoms, and earlier onset of symptoms.[11,20,21]

With the occurrence of environmental and phenological changes, it has become necessary to update, by volumetric method, the concentration of grass pollens in our city.

Methods

The sampling site chosen was the terrace of the Administration Building of the Polytechnic Center of the Universidade Federal do Paraná (UFPR), in Curitiba. The sampler was positioned at a height of approximately 25 meters from the ground. The sampling equipment was the Hirst-type volumetric sampler, SporeWatch Spore Sampler, most commonly used for monitoring and counting pollen around the world[14] (Figure 1).

Externally, the sampler is composed of a wind vane that guides the hole in the direction of the prevailing wind, a protection on top against precipitation, and a base for attachment to the terrace surface. Internally, the sampler has a drum, which is covered with polyester tape and fixed in such a way as to allow it to rotate at a speed of 2 mm/h for 7 consecutive days. The sampling period occurred between the months of January and December 2018, in which approximately 40 weeks were sampled.

The material for particle adhesion was gelatin containing 20% glycerol, 5% agar-agar gelatin, and 0.5% phenol in distilled water for 100 mL spread evenly over the tape with the aid of a pipette. After the gelatin had dried on the tape, it was fixed on the drum of the sampling apparatus with a double-sided tape.

We used Melinex polyester tape 19 mm wide and cut to 48 mm, each corresponding to one day of sampling. Each piece was fixed on a glass slide and stained with basic fuchsin solution for analysis under a Nikon Eclipse E200 optical microscope at 400x magnification.

Morphologically similar grass pollens and pollens from other plants were identified and counted in number of grains/m^3 of air. The intensity of pollen dispersal followed the classification of the National Allergy Bureau (NAB), which is part of the American Academy of Allergy Asthma & Immunology.[22]

Figure 1
Hirst-type, SporeWatch Sampler

A total of 222 days were sampled in the year 2018, corresponding to 60% of the year. All months are represented, and the days without samples are related to technical problems with the sampler or the sample, such as rainy days and holidays.

Table 1
Classification of daily grass pollen dispersal according to the National Allergy Bureau (NAB)[22]

Daily concentration (grains/m^3)	Classification
0	Absent
1 – 4	Low
5 – 19	Moderate
20 – 199	High
> 200	Very high

Results

Bioaerosols were counted using two criteria: 1) total pollen (TP), which includes *Poaceae*; 2) pollen from the *Poaceae* family.

The highest daily concentration of total pollen recorded occurred at the beginning of August, corresponding to 302 grains/m^3. The month of August had eight of the highest daily peaks of total pollen concentration, seven of them higher than 200 grains/m^3. The highest percentage of grasses occurred in March, reaching 29% in relation to the total pollen, and December with the lowest contribution of *Poaceae* pollens, only 0.9% in relation to the TP.

Of all the days sampled in 2018, only 12 did not contain any pollen particles, among which five days were in May and four days were in June. *Poaceae* pollen was found across the year and the highest daily *Poaceae* concentration was 27 grains/m^3 in August and September. The second highest peak was 23 grains/m^3 and occurred on 1 day in the months of February, March, April, and October.

Among the days sampled, 21 days had *Poaceae* concentration higher than 10 grains/m^3 and six days, higher than 20 grains/m^3. In 54% of the samples, corresponding to 120 days of those sampled, no *Poaceae* pollen was observed (Figure 2).

Discussion

This study showed that the distribution of total pollen and *Poaceae* occurred throughout the year, although in varying concentrations. The contribution of *Poaceae* to the total pollen concentration in Curitiba on the annual average was approximately 10% of the sampled pollens. On six days spread over the year, counts were within the limits considered high by NAB.[22]

Despite the extensive distribution of grasses in the city of Curitiba, other anemophilous species have a large participation in the pollen concentration in the city air throughout the year, a fact also observed in the municipality of Caxias do Sul, where *Poaceae* corresponded to 12% of the total pollen.[14]

The first pollen count in the city of Curitiba occurred in 1944 for seven consecutive months, showing that the pollination season of grasses occurred between the months of May and June.[23] The second pollen count was performed in the months of February to August 1960; however, no pollination season of grasses was observed, only for *Cupressaceae*.[24]

It is estimated that pollinosis in the South of the country emerged between the 1970s and 1980s, based on the observation of seasonal allergic rhinoconjunctivitis with intense sensitization to allergic

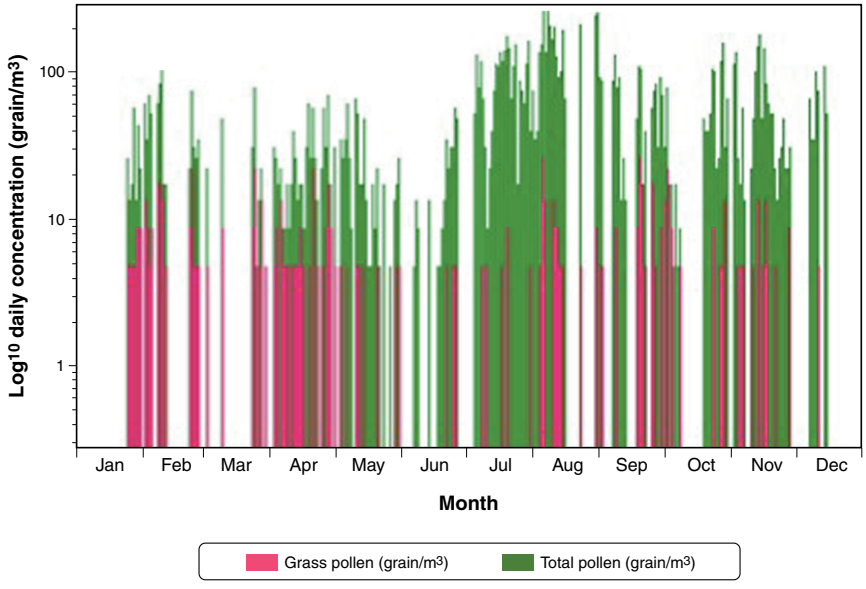

Figure 2
Daily concentration of grass pollen grains (pink) and total pollen (green)

tests with extracts of different grass species, in the city of Curitiba, until then considered an exception in Brazil. Because they occur in the spring months and not in May/June as shown in previous studies, it motivated sampling of airborne pollens in the years 1981/1982, to verify the pollen season of grasses.[5,10,11]

The collection of pollen was done by the gravimetric method using the Durham sampler and the pollen was counted in an area of 1 cm^2. From the results, it was possible to observe two annual peaks of grass pollen concentration, the highest occurring in the second to third week of November and reaching 114 grains/cm^2, and a lower peak of concentration in the month of March and April.

Sampling with the Durham gravimetric sampler was repeated 10 years later in 1991 and it was observed that the peak of grass pollen counts occurred in the second week of November, with 105 pollen grains/cm^2, confirming the spring seasonality and showing that the intensity of grass pollen dispersal had increased in the city of Curitiba.[7,20,21]

The study of pollen concentration in the Southern Region and the relationship with allergic diseases is of high relevance in the context of population health in Brazil. In the present study, it was possible to establish a pollination pattern for *Poaceae* throughout the year in Curitiba. The months between August and April presented the highest concentrations and the total pollen from this period corresponded to approximately 91% of the total grass pollen sampled. The months from May to July had the lowest concentrations and accounted for only 9% of the total *Poaceae* pollen.[8]

In 1982, the highest peak concentration of *Poaceae* occurred in November, followed by smaller peaks in March and January, and near-zero concentrations between the months of July, August, and September.[8] The repeat study in 1991 showed that the peak atmospheric dispersal of grass pollen occurred two weeks earlier, but still in November.[10,11]

In the present study, however, it was observed that the grass pollination season began earlier, in the month of August, but the end of grass pollination remained in the month of April. Therefore, for the year 2018, 36 years after the first collection from Rosario Filho, the grass pollination season was extended for two more months, with the peak concentration also being shifted to the months of August and September. This phenomenon was also noted in Italy, in which grass pollination was measured over 33 years and it was noted that the onset of pollination decreased by -0.4 days/year, with no significant change in the end date of pollination.[9]

Several long-term research studies around the world have studied the correlations between climate change, such as temperature increase, and changes in pollen concentrations in the atmosphere obtained over the years. The introduction of non-native grass species, deforestation, and anthropogenic climate change have likely enabled the spread of *Poaceae* grasses in southern Brazil and the emergence of pollen allergy in these regions.[5,7,20] A recent study on the subject found evidence that among 17 cities in different countries in the Northern hemisphere, 12 had increased annual pollen loads, and in 11 locations the duration of pollination was extended over time, results that demonstrate that the changes are global and independent of latitude.[9,25,26]

Conclusion

The results of this study show the current estimates of pollen concentrations in the capital of the state of Paraná. Therefore, with such data the present research aimed to enable the planning, forecasting, and development of prevention measures to mitigate allergic diseases caused by grasses, the main source of pollinosis around the world.

A limitation of airborne pollen sampling is that it does not allow identification of which species are predominant and at what time of year, because grass pollens are morphologically identical. However, for the clinician this is important, once *Cynodon dactylon* is a species with a wide distribution throughout Brazil and with an allergic sensitization rate similar to *Lolium spp*.

This work should be complemented with further sampling throughout the year in Curitiba to observe whether earlier pollination and a longer distribution and at two concentration peaks, are prevailing. These changes in pollen dispersal serve to alert allergy sufferers in southern Brazil to the possibility of symptoms occurring earlier and longer than previously demonstrated in those sensitized to grass pollen.

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