Title Seasonal behaviour of *Alternaria* airborne spores in Santiago de Chile, Chile

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Seasonal pattern of *Alternaria* airborne spores in Santiago de Chile, Chile (2005–2015 period): first *Alternaria* spore calendar

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Abstract

Since *Alternaria* is an important aeroallergen in temperate areas of the world, this study was undertaken in order to provide the first results obtained about the seasonal regimen of *Alternaria* airborne spores in the atmosphere of Santiago de Chile (Chile), for a period of 10 years (2005–2015), which has led to the construction of the first calendar for the city. Furthermore, the periods of maximum presence of these particles in the air were determined together with those days in which the threshold levels set up for the development of clinical symptoms were reached and/or surpassed. The annual spore integral varied between 4077 spores/m^3 registered in 2013–2014 and 6824 spores/m^3 in 2010–2011, with a main spore season from mid-winter (mid-July/mid-August) to the end of the autumn (June). Daily peaks were mainly detected in spring or autumn seasons but even in winter, although without surpassing 65 spores/m^3 in any case.

(2005-2015 period): first *Alternaria* spore calendar.

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Abstract

Since *Alternaria* is an important aeroallergen in temperate areas of the world, this study was undertaken in order to provide the first results obtained about the seasonal behaviour of *Alternaria* airborne spores in the atmosphere of Santiago de Chile (Chile), for a period of 10 years (2005-2015), which has led to the construction of the first calendar for the city. Furthermore, the periods of maximum presence of these particles

in the air were determined together with those days in which the threshold levels set up for the development of clinical symptoms were reached and/or surpassed.

The annual spore integral (ASIn) varied between 4,077 spores/m^3 registered in 2013-2014 to 6,824 spores/m^3 in 2010-2011, with a main spore season from mid-winter (mid-July/mid-August) to the end of the autumn (June). Daily peaks were mainly detected in spring or autumn seasons but even in winter, although without surpassing 65 spores/m^3 in any case.

Key words *Alternaria*; spore calendar; Santiago de Chile

Introduction

Up to 80 genera of fungi have been associated with IgE-mediated allergic pathology and its clinical impact is presumably related to exposure levels (Rick et al. 2016). Allergic respiratory disease caused by fungi affects both the upper and lower respiratory tracts in people sensitized to them, being *Alternaria* spores a well-known biological contaminant

and a potent source of aeroallergens (Gabriel et al. 2016). In that sense, average daily threshold concentrations between 50 and 1200 spores per cubic meter of air have been established for the triggering of clinical symptoms (Caretta et al. 1992).

Due to their way of life, fungi are ubiquitous and cosmopolitan organisms frequently found in shady and humid areas of forests, as well as on lawns, compost piles and on certain seeds in parks and gardens in urban areas. Although, the seasonal behaviour of said biological particles is conditioned by differences in the vegetal landscape present in the area of influence of the spore trap, as well as by variations in meteorological parameters (Skjøth et al. 2016), which means that their values fluctuate from one year to another and that they cannot be extrapolated even in geographically close areas.

For all this, in recent years, and worldwide, the number of monitoring stations that record the presence of these particles in the air has increased (https://www.zaum-online.de/pollen/pollen-monitoring-map-of-the-world.html). However, the number of works published in this regard is still very limited at present, since studies that cover the full spectrum throughout the year are not frequent, but rather specific works (monthly, seasonal, etc.) of those types of fungal spores of greater interest.

In that sense, although the relationship between the atmospheric content of airborne fungi spores and allergic clinical processes is scientifically proven in Mediterranean areas of the European continent, there is very limited information in this regard in some countries of the American continent, among which figure Chile.

In regard to this country, the Aerobiology, Environment and Health Foundation (FUNDAMAS) has been responsible for the four stations available to the Chilean Aerobiology Network. In Santiago, in association with the Servet Clinic since 1994 and then in conjunction with FUNDAMAS from 2009 to date; in Talca in association with the Catholic University of the Mauleen and then with the University of Talca from 2007

111 to 2011; in Temuco in conjunction with the Catholic University of Temuco from 2006
112 to 2011 and in Valparaíso in collaboration with the Carlos Van Buren public hospital
113 from 1997 to 2011. However, currently, only the Santiago spore trap is operational
114 (http://www.polenes.cl/sitio/default.asp).
115 Considering this city, some aeromicological information was published more than a
116 decade ago (Urrutia et al. 1976; Ibáñez Henríquez et al 2001), but the present work
117 represents the first complete study of the spore spectrum of *Alternaria* airborne spores
118 in Santiago de Chile, including a long data set, since, recently, the analysis of the
119 samples has mainly focused, on the count and identification of the different pollen types
120 (Toro et al., 2015).

121 **Material and methods**

122 Santiago de Chile (33º27'S; 70º38'W) is located at a height of 560 m.a.s.l., in a valley
123 that is flanked to the North and South by two ranges extending from East to West, to the
124 East by the Andes chain (with an average altitude of 4,500 m) and to the West by the
125 Chilean Coastal Range (with an average altitude of 1,500 m). It is the capital of the
126 Republic of Chile and the urban part of the Metropolitan area (which represents over
127 40% of the country's inhabitants) is divided into 34 communities, being Providencia
128 (the location of the monitorization point) characterized by a significant presence of
129 businesses together with residential areas. The climate is Mediterranean with a mean
130 temperature of 14.0ºC, ranging from 2.0ºC to 35.0ºC (Toro et al., 2015).
131 The aeropalynological monitoring was carried out from June 27th, 2005 to April 26th,
132 2015, using a Hirst-type trap, Burkard model, placed on the roof of the Clínica Miguel
133 Servet building (33°25S, 70°37'W, 600 m.a.s.l.), at the Comuna de Providencia in
134 Santiago de Chile (Chile), at a height of XX m above the ground level, according to the
135 guidelines established by the World Allergy Organization (Hasnain et al., 2007).

The pollen samples were processed and analyzed using a longitudinal "read", being this methodology granted a national and international certification by the Chilean Society of Allergy and Immunology and the American Academy of Allergy Asthma and Immunology, respectively. Different keys and morphology works were used to the identification of the moulds (Grant Smith 2000, among others).

The seasonal behaviour of atmospheric spore concentrations was evaluated defining the main spore season by the method proposed by Nilsson & Persson (1981), which calculates the start of this period as the date by which 5% of the total year spore record was registered, and the end by the 95% captured. Being Santiago de Chile located in the South Hemisphere, the beginning of the year has been considered in July, coinciding with winter time. In that sense, information about the characteristics of the MSS of the different years is shown in Table 1. Furthermore, the mean annual behaviour for the 2005-2015 period has been graphically represented according to a 5-day running mean (Fig. 1a), together with the annual periods in which the daily threshold level of 50 spores/m^3 established (Caretta et al. 1992) was surpassed (Fig.1b).

The spore calendar construction (Fig. 2a) was based on Spieksma's model (Spieksma 1991), transforming 10-day mean concentrations into eleven classes, according to a minimum 10-day mean equal to or greater than 1 spore/m^3 (Fig. 2b).

Results and discussion

Characteristics of the Main Spore Season

The annual spore integral (ASIn) varied between 4,077 spores/m^3 registered in 2013-2014 to 6,824 spores/m^3 in 2010-2011 (Table 1), concentrations similar to the ones registered in some European urban cities (Skjøth et al. 2016), although, the main spore season is much longer (Fig. 1a), from mid-winter (mid-July/mid-August) to the end of the autumn (June), probably due to the regular record of milder temperatures typical of

Mediterranean sites (Skjøth et al. 2016). However, it is remarkable that this pattern seems to have changed within the city over time, since it is different from the one observed by Ibáñez et al. (2001), in which there was a clear predominance of *Alternaria* fungal spores during the autumn period, mainly in April.

As in the South of Europe, where the highest concentrations were registered in May-June or September-October (Skjøth et al. 2016), daily peaks were mainly detected in spring time (October 13th 2012, 22nd 2006; November 18th 2012 and December 3rd 2014) and autumn season (April 4th 2007, 17th /18th 2011; May 3rd 2008, 23rd 2009 and June 3rd 2006, 5th 2012), but even in winter (August 28th 2009; September 9th 2013), although without surpassing 65 spores/m^3 in any case (Table 1), in contrast with Ibáñez et al. (2001) that reported daily concentrations of even 179 spores/m^3.

Related to that, the number of days in which the threshold value considered (50 spores/m^3) was surpassed was very scarce (1 day in 2009-2010 and 2014-2015 periods; 3 days in 2010-2011,4 days in 2007-2008 and 7 days in 2066-2007) (Fig. 1b), which might be in concordance with the uncommon sensitization to *Alternaria* observed (Rona et al. 2008).

Spore Calendar

It should be highlighted that *Alternaria* spores reached just class 5 (24-49 spores/m^3) as its highest exponential class, similar but lower than in other European Mediterranean locations in which class 6 was reached (Fuentes Antón et al. 2019), with a very irregular register throughout the annual periods. Nevertheless, the most frequent one was class 4 (12-23 spores/m^3).

With respect to the permanence in the atmosphere it is important to point out that *Alternaria*, reached 10-day means greater than 1 spore/m^3 along the whole annual periods except for 2007-2008 (the second 10-day period of January), 2010-2011 (the

first one of January) and 2014-2015 (from the second 10-day period of May until the end of June) (Fig 2).

As conclusion, *Alternaria* maintained an irregular distribution pattern during the year, being more abundant in the summer and autumn seasons. However, it did not reach high daily concentrations. In that sense, a more recent clinical research would be recommended in order to determine if the threshold value established is adequate considering clinical aspects.

Deeper research is also necessary to study the influence of local vegetation structure and the meteorological parameters on *Alternaria* airborne spores behaviour in terms of climate change.

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Figures captions

Fig 1a. Seasonal variation of *Alternaria* (2005-2015 average; 5-day running mean). **b.** Number of days in which *Alternaria* threshold value (50 spores/m^3) was surpassed.

Fig 2a. *Alternaria* **s**pore calendar for Santiago de Chile. **b.** Exponential classes proposed for the construction of the *Alternaria* spore calendar.