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Study of the reproductive phenology of *Araucaria angustifolia* in two environments of Argentina: Its application to the management of a species at risk.

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Abstract

Araucaria angustifolia is an extinction-threatened arboreal anemophilous conifer species native to Argentina and Brazil. A progressive scarcity of seeds that affects the natural regeneration and the development of new plantations has been observed. Taking into account that seed formation depends on pollination, pollen productivity was analyzed and the influence of the climate on the different reproductive phenophases evaluated. Two Argentine populations were compared: San Antonio (SA), located in the subtropical province of Misiones within its natural area, and 25 de Mayo (25M) in the temperate province of Buenos Aires. Gravimetric pollen traps were used during 2014, 2015, and 2016. It was found that the average of annual pollen productivity in 25M doubled that of SA (9440 and 5291 pollen cm^{-2} year^{-1}), and that seed productivity was 10 times higher in 25M with 104 seeds per cone compared to 12 in SA. High maximum summer temperatures were favorable to the induction of reproductive structures, low minimum temperatures in August favored the maturation of pollen grains, and precipitations in the main month of pollination reduced the amount of pollen. The weather in Buenos Aires Province is more favorable for the production of reproductive material. In this context, *ex situ* seed banks offer the chance of enhancing and restoring native forests and *in situ* reforestation.

Keywords: Paraná pine, pollen, seeds, temperature.

SA: San Antonio, 25M: 25 de Mayo, PI: pollen influx.

Introduction

Araucaria angustifolia (Bert.) O. Kuntze, also known as the Paraná pine, is native to southern Brazil and northeastern Argentina. This species was much appreciated for the high quality of its wood in the last century. Today, according to the International Union for the Conservation of Nature and Natural Resources (IUCN) (Thomas, 2013), it has become a critically endangered species due to its indiscriminate exploitation in the natural forests of Argentina and Brazil, without regard to reforestation and its very slow growth rate compared to the exotic forestal species of *Pinus* or *Eucalyptus*. The situation of this species is even more critical, since there has recently been a decrease in seed production that has affected the supply of reproductive material from this native forest species, the most wildly cultivated in the country (Fassola et al., 1999).

The Paraná pine is an anemophilous dioecious tree, a shade-intolerant species that dominates the upper strata of the native forest, currently occupying 3% of its area of origin. According to studies conducted with Brazilian populations, the reproductive cycle of *A. angustifolia* lasts around 30 months. The female cones and the male strobili develop in November and pollination occurs in September-October of the following year. Then, it takes 20 months more for seeds to mature (Anselmini *et al.*, 2006).

Pollination is the necessary phenologic phase for fertilization and seed formation, and its limitation may be associated with the low number of pollen grains available for fertilization and thus a low cross-pollination possibility. On the other hand, in general terms, pollen productivity is associated with seasonal weather conditions prior to pollen release (Caramiello *et al.*, 1990, Latorre, 1999). European aerobiologists have found positive relationships between the mean temperatures of the days before pollination and the onset of the pollination period, specifically in alders, elms, pines and birches (González Minero *et al.*, 1999), and also in olive (Galán *et al.*, 2001). Particularly, in a two-year preliminary study undertaken in a population of *A. angustifolia* in Misiones (Argentina), a decrease in the number of microsporophylls was observed in the year with the highest minimum temperature during its formation period in the first months of the year and in the winter months (June, July and August) previous to pollination (Caccavari *et al.*, 2000). On the other hand, consistent with studies performed in Brazil by Anselmini *et al.* (2006), the increase in temperature and rainfall in November and December favors the formation of reproductive structures; and between December and April, maturation of seeds.

To analyze the effect of meteorological variables on the different reproductive stages of this species, a study comparing the phenology of *Araucaria angustifolia* growing in two regions of Argentina under different climatic conditions was conducted. Aerobiological data from San Antonio (SA), Province of Misiones (plantations for *in situ* conservation) and 25 de Mayo (25M), Province of Buenos Aires (plantations for *ex situ* conservation) were analyzed to estimate the production of reproductive material. The purpose was to determine how the climatic/meteorological conditions influenced pollen productivity of *A. angustifolia* and to analyze how seed production was affected. The relationship between both reproductive phenological events will allow to define a model to estimate seed harvest in advance, and to develop effective conservation management strategies for this critically endangered species.

Since one of the salient factors affecting seed formation is pollen availability for fertilization, it was proposed that high winter temperatures prior to pollination negatively affected pollen production in line with Latorre *et al.* (2015). This hypothesis, along with global temperature increase, which is also detected in northeastern Argentina, would explain the progressive reduction in seed crop observed during the last years for *A. angustifolia* in its place of origin. Therefore, the effect of temperature was evaluated by comparing both sites during the initial and final stages of the reproductive cycle: strobilus formation and maturation of seeds.

Materials and methods

Description of the study areas

Studies on the reproductive phenology of *Araucaria angustifolia* were carried out in two Argentine provinces on plantations of the National Institute of Agricultural Technology (INTA), one located in the Manuel Belgrano Field (Campo Anexo Manuel Belgrano, CAMB) near San Antonio locality, Misiones Province (study site: SA, 26º 3' S, 53º 46' W, 544 m a.s.l.), and the other in 25 de Mayo Forest Station (Estación Forestal 25 de Mayo) near 25 de Mayo city, Buenos Aires province (study site: 25M, 35º 30' S, 60º 07' W, 58m a.s.l.) (Fig. 1). Both sites presented individuals of similar reproductive age, and not over-mature (between 50 and 60 years old). SA plot density is 332 trees ha^{-1} and that of 25M is somewhat higher with 468 trees ha^{-1}. Nevertheless, since some trees in 25M do not reach the upper canopy, its reproductive development is restricted, and so both plots were considered with similar potential reproductive yields. In line with Barrera *et al*. (2002), canopy structure is the variable that best relates to pollen production.

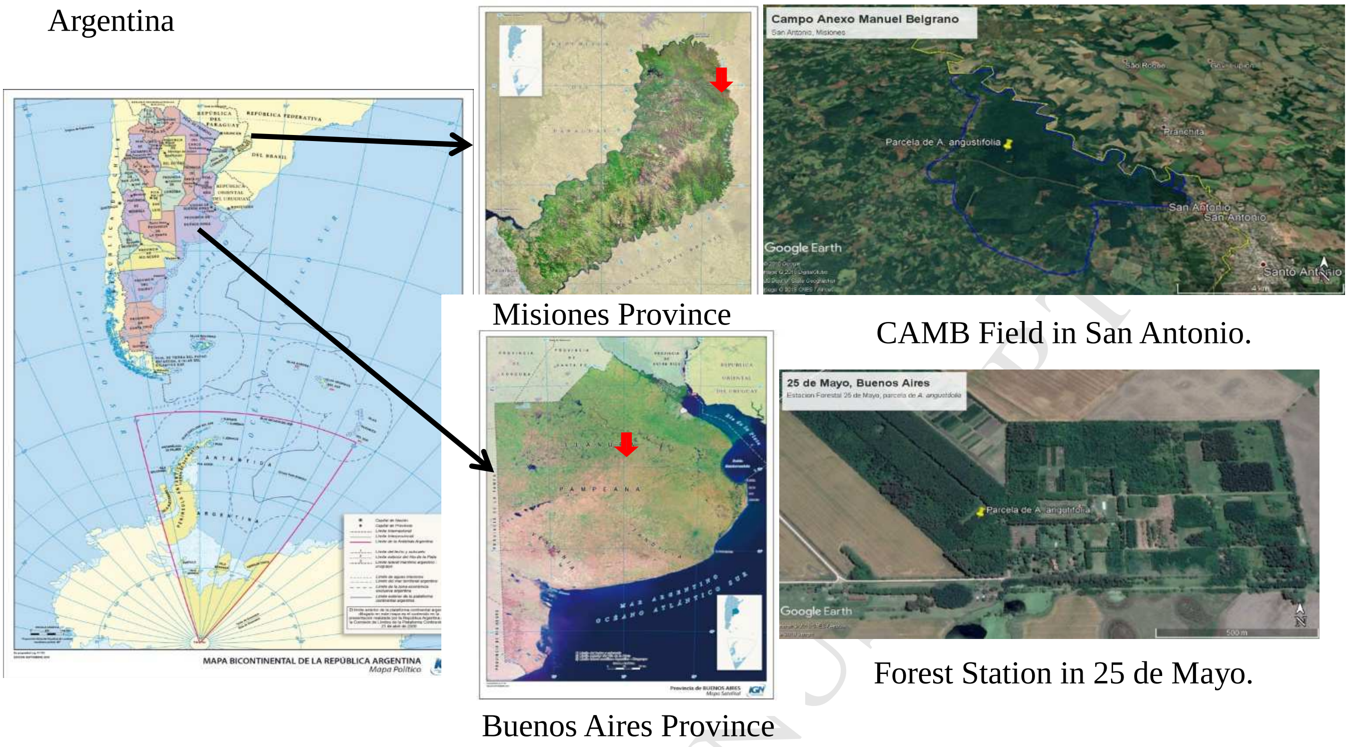


Figure 1. Study sites: Manuel Belgrano Field (Campo Anexo Manuel Belgrano, CAMB) in San Antonio, Misiones, and Forest Station (Estación Forestal 25 de Mayo) in 25 de Mayo, Buenos Aires, Argentina, both belong to INTA (National Institute of Agricultural Technology). Both meteorological stations are located near the *A. angustifolia* plot, within the study sites (National Geographical Institute (http://www.ign.gob.ar) and Google Earth).

According to the Köppen classification, SA, Misiones, has a humid subtropical climate without dry season (Cfb). Mean temperatures range from 25ºC in summer (January, February, March) to 14ºC in winter (June, July, August). It receives an annual rainfall between 1600 and 2000 mm, distributed throughout the year (although rains witness a drop in July and August). San Antonio is located within the Paranaense phytogeographic province (Cabrera, 1994). The climate in 25M, Buenos Aires, according to Köppen classification, is warm temperate with winter rains (Csb), with two well-differentiated periods: a cold one that extends from late April to late September, and a warm one that extends from October to March. The mean temperature in summer is 23.2°C and in winter 7.3°C; the average annual precipitation is 910 mm, and minimum rainfall occurs between May and August. 25 de Mayo area belongs to the Pampeana phytogeographic province (Cabrera, 1994).

Both study sites also differ regarding soil and topography. SA is located in a mountainous area with low elevations averaging 500 m a.s.l., while 25M is in a plain with altitudes of 50 m a.s.l., in average. Brazilian natural forests are located between 400 and 2300 m a.s.l. SA has acid and well-developed red soils, drained and clayey with fine texture and iron oxide, low amount of nutrients and good physical conditions for root growth, being classified as orthoxic Kandihumultes. In 25M, soils are alkaline hydromorphic with sandy texture of Brunizen type (typical Argiudol) and a textural B horizon, well supplied with nutrients.

Pollen Sampling and Analysis

An aerobiological sampling was carried out with Tauber traps (Tauber, 1974), which collect airborne pollen by gravity. The sampler device consists of an appropriately-sized vessel to avoid rain water overflow and the re-suspension of particles already deposited. Its main feature is its lid with an opening of 5 cm in diameter through which particles suspended in the air fall by gravity, and this is the sedimentation surface. The lid has aerodynamic features that prevent air flow generation next to the sedimentation area, which could compromise the sedimentation process. These field samplers were placed below the tree canopy at 1.5 m above the ground in the center of the plantation. Pollen monitoring of *Araucaria angustifolia* in SA and 25M was extended throughout the reproductive period (from August to December) during 2014, 2015, and 2016.

Tauber samples were processed following the standard procedure (Faegri & Iversen, 1992), though without performing acetolysis. *Lycopodium* spores were added as foreign markers in order to calculate absolute pollen values (Stockmar, 1971). Each *Lycopodium* tablet contains a known number of spores (Batch Nº 483216). A Leica DM 500 optical microscope with digital camera was used for determination and counting. To accurately represent the entire sample, “count intervals” were established using one *Lycopodium* spore as the unit of measure. In each interval the number of *Araucaria* pollen grains found were counted. This procedure continued until a minimum of 200 spores of *Lycopodium* was reached. For each spore counted, the number of recorded *A. angustifolia* grains varied between 1 and 15. The sample size was considered appropriate (representative of the total and comparable between sites and years) when the number of *A. angustifolia* grains that appeared for each spore of *Lycopodium* counted (grains/spores ratio) reached a stable value, even if the count continued. Values up to the first 100 *Lycopodium* were discarded, since ratios fluctuated. Finally, the mean of the counts between 100 and 200 *Lycopodium* spores was set as the sample value. The percent coefficient of variation (CV%) (Daniel, 1991) for the 100-200 interval data was less than 4% for most samples, reaching 11% in one sample.

Then, the absolute values of total pollen abundance (Pollen Influx: PI) were calculated (Hicks & Hyvärinen, 1999). This value was expressed as the number of pollen grains deposited on a unit surface area (pollen cm^{-2}), for each year and site, and was calculated as follows:

Total pollen = (total *Lycopodium*) x (counted pollen) x (counted *Lycopodium*)$^{-1}$

PI = (total pollen) x (deposition surface) $^{-1}$

Sample processing and analysis were carried out in the Department of Biology, Faculty of Exact and Natural Sciences of the Universidad Nacional de Mar del Plata.

Seed Sampling

Between 30 and 34 seed cones were collected from at least 10 trees following transects every 10 meters in the studied plots. Generally, cones with seeds were collected from the ground beneath the canopy projection area. When it was possible, they were obtain directly from the species, by climbing the tree to remove them. Seed count per cone was done, and the mean number of seeds per cone was calculated for each site and year. Because of their potential viability, only full seeds were considered.

Weather Information

To compare sites and establish the atmospheric conditions that affected pollen productivity and thus pollination and seed formation, data from the INTA Agrometeorological Stations were used. Variables included: mean temperature, maximum temperature, minimum temperature, relative humidity, precipitation and wind speed reported from November 2013 to April 2017. Also, the anomalies of the maximum and minimum temperatures were calculated, i.e., the difference of these variables with respect to the historical average (National Meteorological Service).

Data Analysis

To determine the weather conditions related to PI, Spearman's rank correlation coefficient was calculated (Daniel, 1991) between pollen and weather variables. Once the correlations were determined, forward stepwise multiple regression analyzes were performed with STATISTICA software (StatSoft, Inc. 1984 - 1999), in order to establish the variables that could mostly affect the amount of pollen recorded and seeds collected. To do so, the most relevant meteorological variables in each phenological stage were used:

a) Before pollination (to evaluate the effect on strobili formation and on pollen grain maturation): maximum temperatures in November, maximum temperatures in December, maximum temperatures in January, maximum temperatures in February, mean temperature of the maximum temperatures from November to December, mean of maximum temperatures of each month during the summer period (December, January, and February), minimum temperatures in June, minimum temperatures in July, minimum temperatures in August, average winter minimum temperatures (June, July, and August), and mean temperatures in August, for each year under analysis and study site.

b) during pollination (to evaluate the instantaneous effect on the suspended grains): precipitation and wind speed of September and October;

c) during seed formation: maximum, minimum and mean temperatures from December to April, and PI data of each year and site.

Results

Pollen production of *A. angustifolia*

From the comparative analysis of *A. angustifolia* carried out in 2014, 2015, and 2016, it was detected that 25M yielded bigger pollen production than SA (between one and three times higher), and also greater variability between years (Fig. 2).

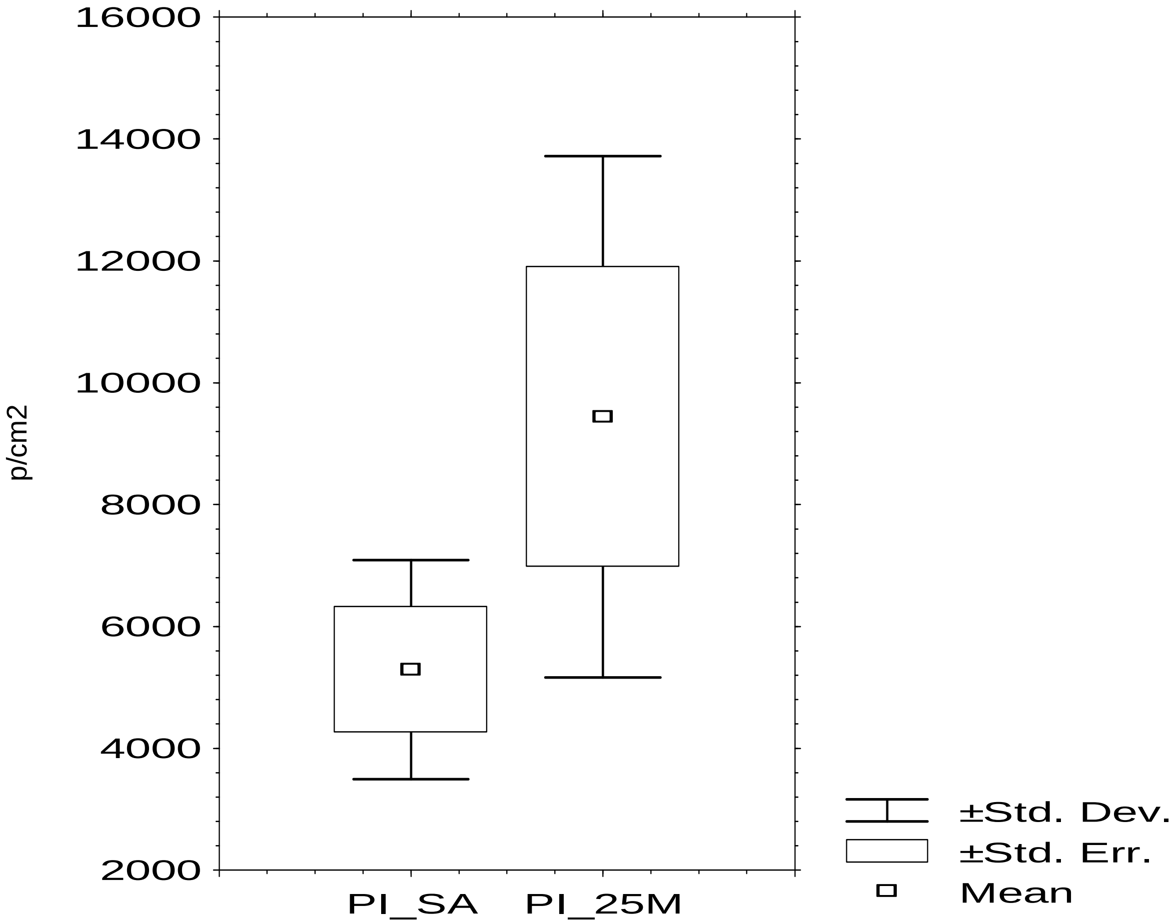


Figure 2. Representation of the mean, standard deviation (Std. Dev.) and standard error (Std. Err.) for Pollen Influx (PI) from all the studied years in each site (SA and 25M), expressed as the number of pollen grains deposited on a unit surface area (p cm^{-2}).

During the first year, 2014, PI value was 39% higher in 25M than in SA (12165 and 7367 pollen cm^{-2}). In 2015, such difference was even greater (64% more in 25M with 11645 pollen cm^{-2} as compared to 4238 pollen cm^{-2} in SA). By contrast, in 2016, the difference reached only 5% (25M with 4510 pollen cm^{-2} and SA with 4267 pollen cm^{-2}).

At both sites, a similar trend was perceived with a decrease in the amount of pollen produced over time, although the rate was lower in SA (Fig. 3). In 25M production decreased very little (4%) between 2014 and 2015, but the difference between 2015 and 2016 (61%) was significant. In SA, pollen production decreased by 42% between 2014 and 2015, though between 2015 and 2016, differences were not significant.

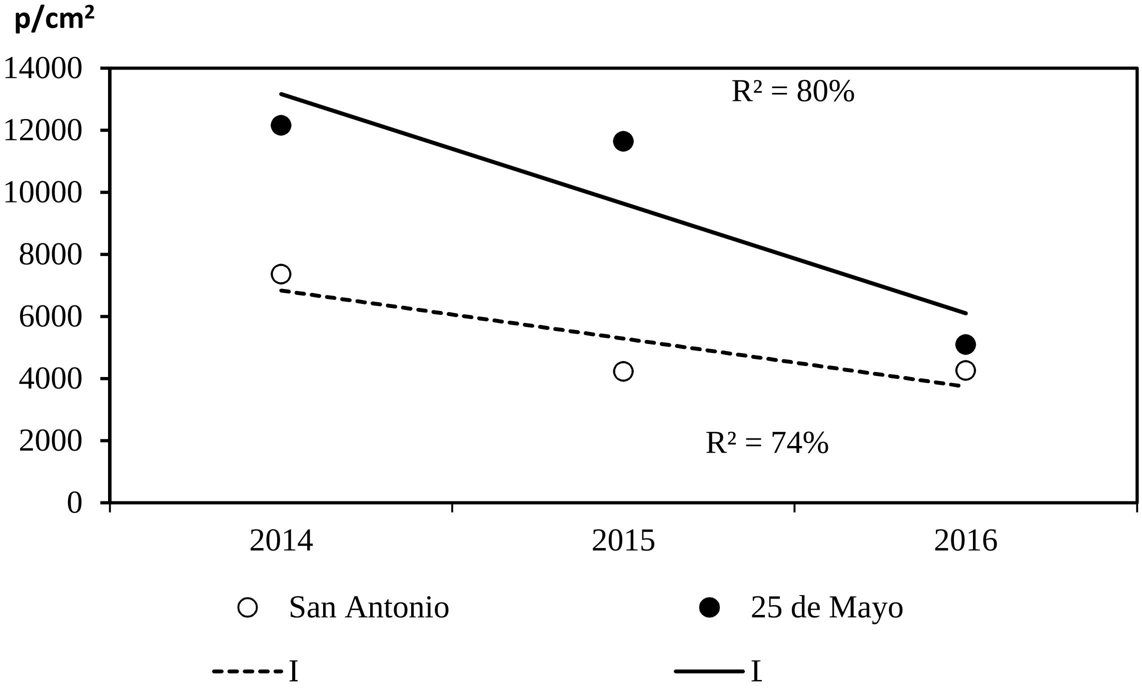


Figure 3. Pollen productivity trend of each site over time. R^2: adjustment to the straight line. p/cm^2: number of pollen grains deposited on a unit surface area.

Production of *A. angustifolia* seeds

The data available to compare seed production between sites and years corresponded to seeds collected in 2016 and 2017 from the 2014 and 2015 pollination periods, respectively. The amount produced per cone in 25M was on average 104 seeds, 10 times higher than in SA with an average value of only 12 seeds per cone. The variation between sites was greater than the variation between years. The differences between years indicated the same trend in both sites, with an increase in production from 2016 to 2017; the increase was five times greater in SA and doubled in 25M (Fig. 4).

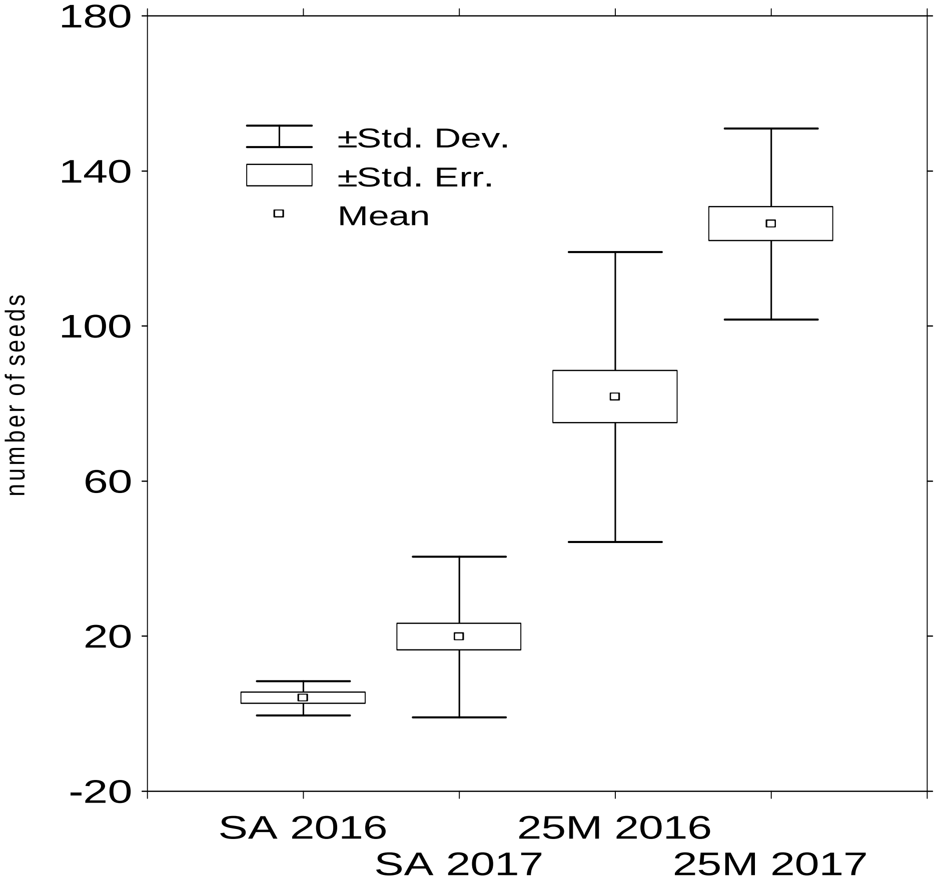


Figure 4. Representation of mean (Mean), standard deviation (Std. Dev.) and standard error (Std. Err.) for the number of seeds per cone in each site (SA: San Antonio and 25M: 25 de Mayo) and year.

Even though the amount of pollen decreased from 2014 to 2015, this inter-annual difference in 25M was low as compared to the values registered in 2016. In SA, the variability in the number of seeds per cone measured by its dispersion with respect to the mean was so great in 2017 that it included the variability of the previous year. As a consequence, the differences between years would not be significant. Figure 5 depicts both sites clearly differentiated by their productivity in both reproductive events: more pollen and more seeds were found in 25M.

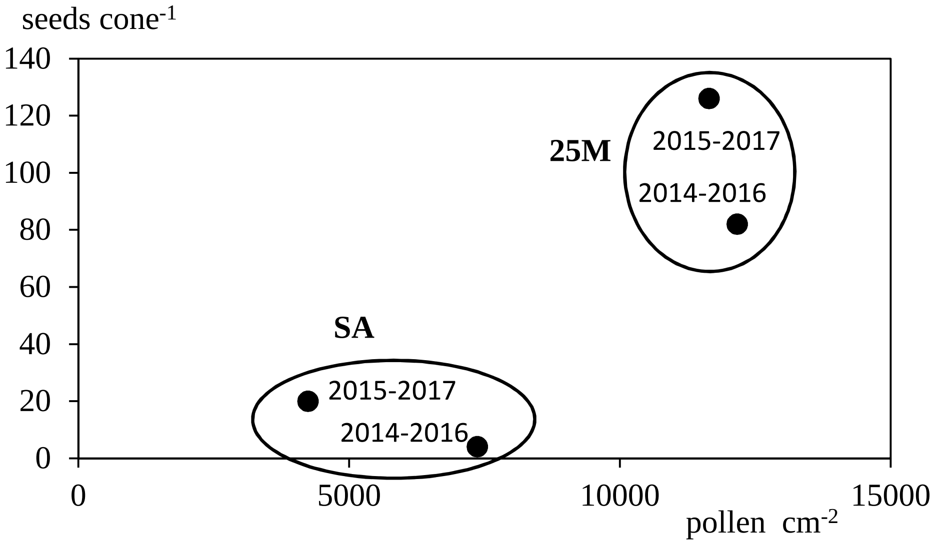


Figure 5. Number of seeds per cone (2014 or 2015) based on the pollen produced (2016 or 2017) at each site. SA: San Antonio and 25M: 25 de Mayo.

Climatic-meteorological conditions prior to pollination

The climatic-meteorological conditions prevailing during male and female cone development, and during the formation and maturation of pollen grains were analyzed. The differences in terms of temperature between sites were established.

The monthly values of the period 1981-2010 of the mean maximum temperatures in 25M were higher as compared to those of SA during December, January and February, and slightly higher also in November. During these months, the androstrobili and ginostrobili developed (Fig. 6).

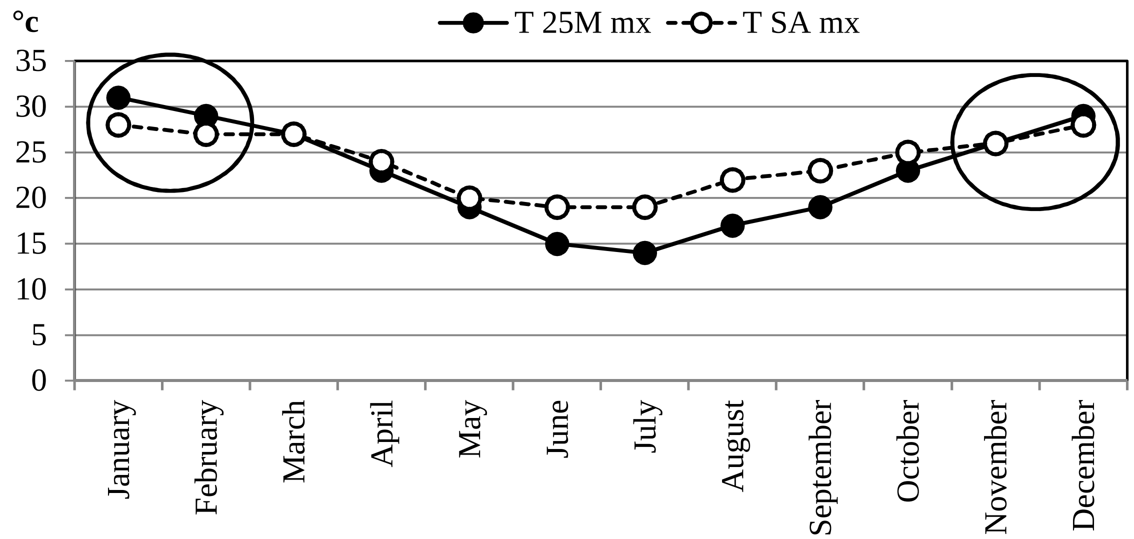


Figure 6: Monthly mean of maximum temperatures (Tmx) for the 1981-2010 period for San Antonio (SA) and 25 de Mayo (25M). The circles highlight the differences between sites regarding the months of reproductive structure formation.

With respect to minimum temperatures over the last 20 years, 25M accounted for lower values than SA in every month of the year (Fig. 7). These differences were greater during June, July, and August, months prior to pollination.

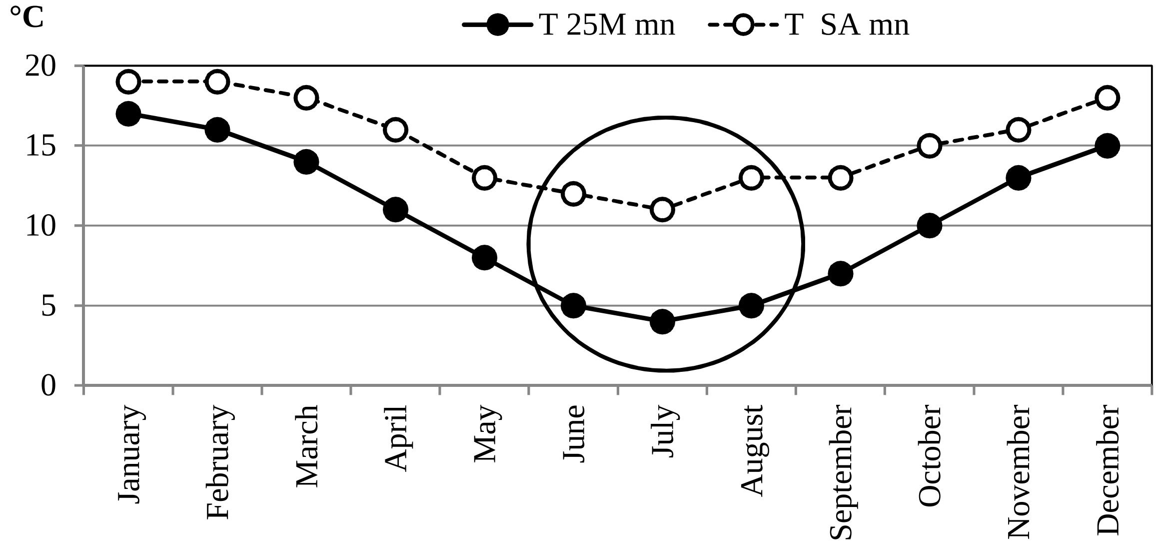


Figure 7: Monthly mean of minimum temperatures (Tmn) for the 1981-2010 period for San Antonio (SA) and 25 de Mayo (25M). The circles highlight the differences between the sites in the months before pollination.

When monthly temperatures in both sites were compared during the study period, it was detected that the maximum temperatures in 25M were higher in the summer months (values less than zero). The opposite occurred regarding the differences in minimum temperatures, which were above 0 in all the months of the year, i.e., higher in SA than in 25M. The highest values of differences in minimum temperatures were recorded in the winter months, prior to pollination (Fig. 8).

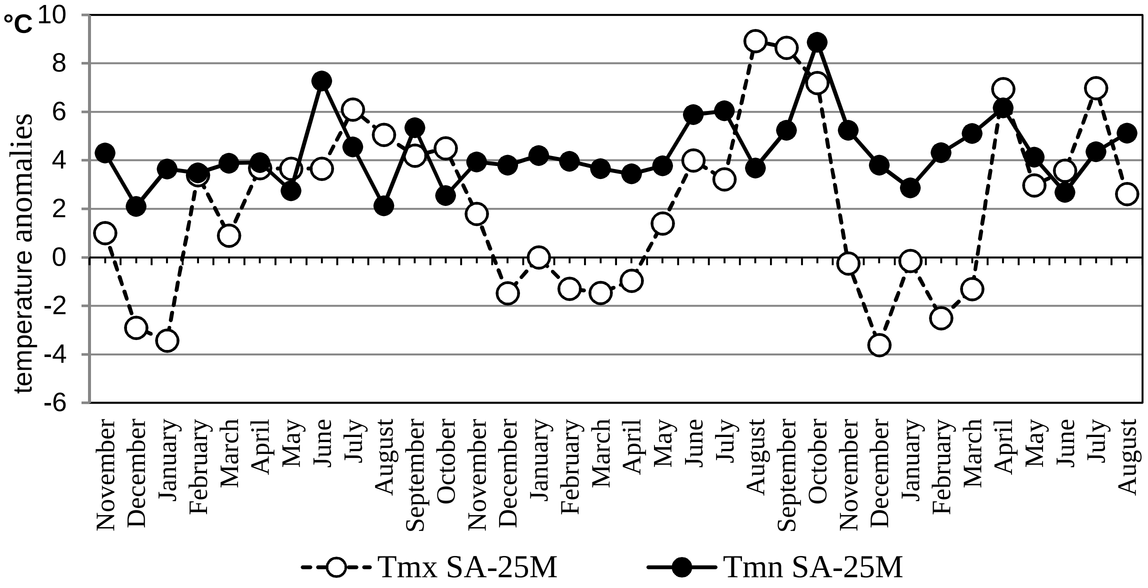
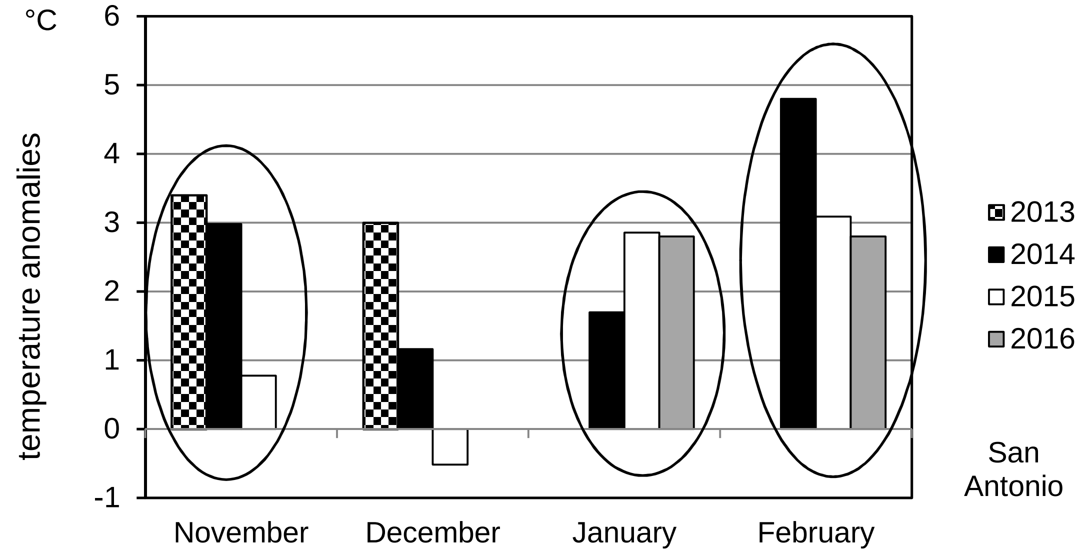


Figure 8: Differences of the maximum (Tmx) and minimum temperatures (Tmn) between San Antonio and 25 de Mayo (SA-25M) for the period November 2013-August 2016. Positive values indicate that the data of the variable are higher in San Antonio.

During the study period the maximum temperatures between November and February exceeded the values of the period 1981-2010 in both places. Nonetheless, it was noticed that maximum temperature anomalies in November, January, and February prior to 2014, 2015 and 2016 pollen years, were higher in 25M as compared to the anomalies in SA, while in December prior to those same pollen years 2014, 2015 and 2016, the maximum temperature anomalies in 25M were higher with respect to SA (Fig. 9).



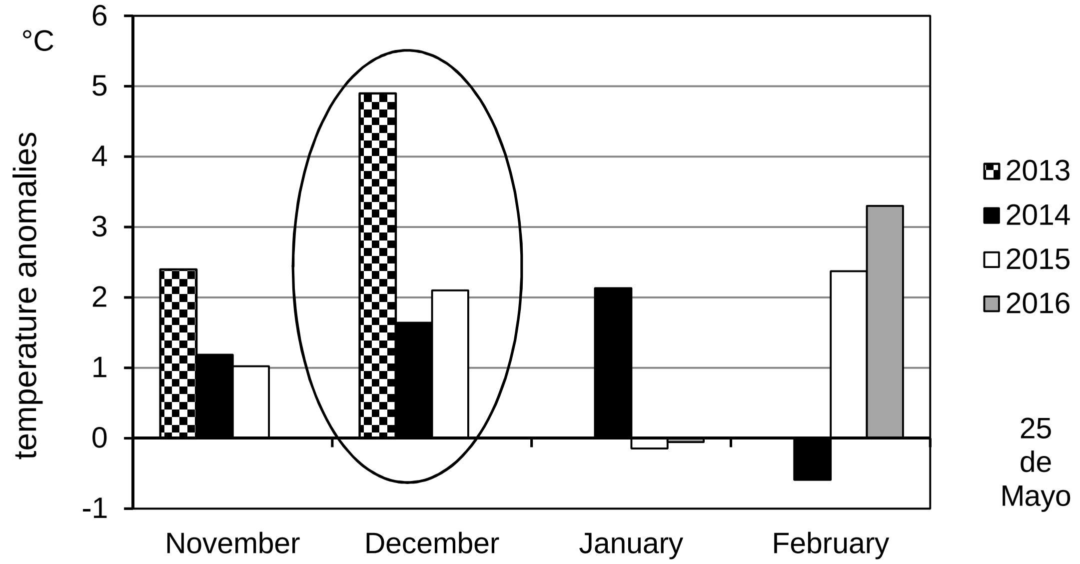


Figure 9: Difference between the maximum temperatures in each year under study and weather values (anomalies) in the months of formation of the female and male cones in San Antonio and in 25 de Mayo. The ellipsis highlights differential months.

Minimum temperatures in SA exceeded 10ºC on average, a value higher than that of the period 1981-2010 in the winter months. Nonetheless, in 25M differences were not significant with respect to the climatic values and always lower than 10ºC (Fig. 10).

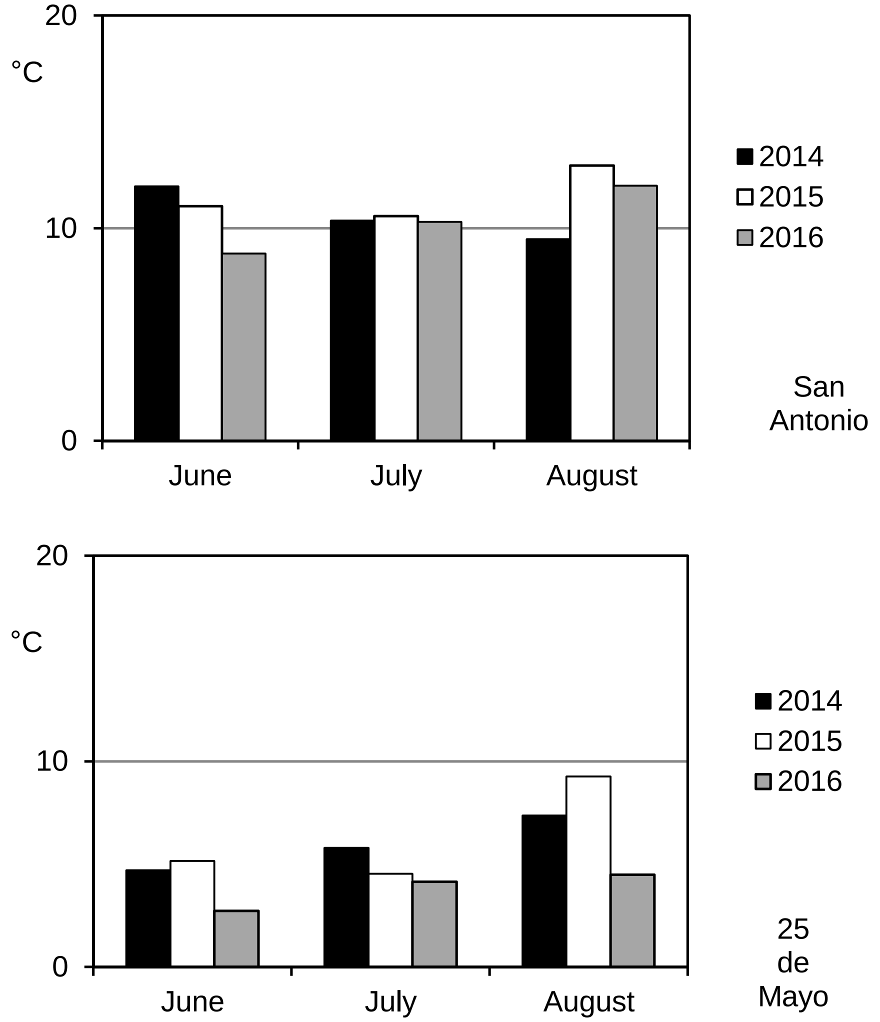


Figure 10: Difference between the mean of the minimum temperatures in each year under study for San Antonio and 25 de Mayo in the winter months prior to pollination.

Analysis of biological and meteorological variables

The Spearman correlation coefficient by ranges (rs) between PI and the meteorological variables was calculated during the different phenological reproductive stages of *A. angustifolia* (Table 1). Maximum summer temperatures (December-January), the station during which androstrobili and ginostrobili develop, correlated positively with PI. The minimum temperatures before anthesis and during the formation-maturation of pollen grains (June-August) were negatively correlated with PI. With respect to the period of pollen emission-collection, the variables that best correlated with PI were wind speed and rainfall in October ($p < 0.05$), in a negative way.

Table 1: Spearman correlation (rS) between annual pollen and meteorological variables during the different reproductive phenophases of *A. angustifolia*. p: significance values.

Annual Pollen	**rs**	**P**
December maximum temperatures	0,71	0,11
January maximum temperatures	0,39	0,43
Maximum summer temperatures	0,77	0,07
June minimum temperatures	-0,37	0,47
July minimum temperatures	-0,48	0,32
August minimum temperatures	-0,66	0,15
October rainfall	-0,89	0,018
Winds in October	-0,89	0,018

When analyzing the dependence of annual pollen on the selected atmospheric variables, a dependency relation was observed with January maximum temperatures and August minimum temperatures (conditions prior to pollination), as well as with the wind speed and precipitation in October, negative in the case of the latter (conditions during emission) (Table 2).

Table 2: Regression coefficients (B) and significance values (p) of the meteorological variables for the registered pollen values. The adjustment to the linear equation was 99.9%.

	B	**p**
Intercept	-12774,31	0,0872
Wind speed in October	3763,72	0,0169
October rainfall	-66,89	0,0236
January maximum temperatures	582,53	0,0592
August minimum temperatures	200,49	0,0681

The number of seeds was correlated with: maximum temperatures between the months of December and April during maturation of seeds (rs = 0.8, p = 0.19), and the amount of pollen recorded two years before harvest (rs = 0.6, p = 0.4).

Discussion

This is the first aerobiological study to compare the reproductive phenological development of *A. angustifolia* growing in two different regions of Argentina: Misiones (northeast) and Buenos Aires (center-east) provinces. The amount of pollen of *A. angustifolia* recorded in each site represents the annual pollen productivity of this species during the study years 2014, 2015, and 2016. The location of the aerobiological sampler under the tree canopies was the most appropriate to estimate that pollen variable (Latorre *et al.*, 2013).

The studied sites showed significant environmental differences: SA has a subtropical climate and is located within the natural distribution area of the species although in the southern margin; while 25M presents a temperate climate and is situated outside the natural area, 1500 km to the south. SA was the least productive site as compared to 25M in all the years of study. The results derived from this work allowed us to infer that the differences observed in the amount of pollen were mainly explained by the meteorological differences between sites that affected productivity.

In particular, it was observed that the atmospheric conditions differentially affected the stages of the species reproductive cycle or phenophases.

Studies conducted in Brazil by Anselmini *et al.* (2006) concluded that an increase in temperature during the summer months, especially November and December, induced the formation of strobili in this species. In Argentina, the results obtained by comparing both sites with different climates, support this hypothesis. In particular, in 25M maximum temperatures were recorded in December (also in January and February), higher than those in SA during the years under analysis. This favorable condition in 25M at this stage of the reproductive cycle would explain in part the pollen differences observed between sites.

Previous results obtained by Latorre *et al.* (2016) in SA from atmospheric monitoring with a Hirst type volumetric sampler (Hirst, 1952) placed under the tree canopy, indicated that high pollen productivity was followed by two consecutive years of pollen progressive decrease. A cycle that repeated itself for two consecutive periods during the six years of the study. Even though cyclical endogenous rhythms have been described in arboreal species, where years of high productivity alternate with years of low productivity (Nielsen *et al.*, 2010), annual pollen of *A. angustifolia* was found to correlate significantly with August temperatures, in a negative way. On the other hand, and according to Fassola (2005), winter high temperatures prior to pollination negatively affect the formation of microsporophylls and, therefore, affect the amount of pollen. In this study, when the weather conditions of both sites were compared during the months in which pollen grains were maturing, the minimum temperatures in June, July and August were in average 5ºC lower in 25M relative to those in SA in all the years analyzed. Moreover, the SA mean minimum temperatures in said months were always higher than 10ºC, while those in 25M never reached this value. According to Caccavari *et al.* (2000), minimum temperatures should not exceed 10ºC during pollen grain formation and maturation, due to their negative impact. Therefore, based on the results achieved and supporting the hypothesis stated, low temperatures prior to pollination favor pollen productivity in 25M or high winter temperatures in SA have a negative influence, leading to low pollen production, or both.

The amount of pollen recorded in 25M during 2016 was lower compared to that registered in previous years in this site. Analyzing the minimum temperatures in the months before pollination, during June 2016, minimum temperatures were below zero. These frosts could account for the decrease in the amount of pollen registered particularly in that year. Despite the fact that relatively low winter temperatures seem to favor pollen production, frost periods could have a deleterious effect on grain formation and maturation. According to Barlow *et al.* (2015), the greatest impacts of frosts on wheat pollen production are associated with sterility and the abortion of grains around anthesis, yielding a decrease in grain number.

The instantaneous negative effect of rainfall on airborne pollen was evident during the month of maximum pollination, October, the rainiest month in Misiones (Peternel et al., 2004; Silva *et al.*, 2014). Wind speed was negatively associated with pollen counts. The analysis of *A. angustifolia* hourly airborne pollen pattern showed a nocturnal increase in pollen concentration (Latorre *et al*., 2013) related to atmospheric stability (Giostra *et al*., 1991). This phenomenon occurs at night, in the absence of wind and when thermal inversion takes place, since the air next to the ground cools faster than the upper air preventing the hot air between two cool air layers from moving and so particles from being transported. On the other hand, pollen grains do not fall from tree crowns (Boi & Llorens, 2008) because of the air currents, leading to a decrease in pollen sedimentation. In this regard, studies are being conducted to evaluate the factors that influence wind-dispersed *A. angustifolia* pollen (Latorre *et al.,* 2014), and gain further insight into the distance reached by the pollen grains.

The joint analysis of the atmospheric conditions that affect pollen production in the different stages of the reproductive cycle of *A. angustifolia* indicated that the factors explaining the amount of pollen recorded would be: the maximum temperatures in January during strobili development, the minimum temperatures in August during pollen grain formation, and rainfall and wind in October during pollen release.

Aerobiological studies have reported a close relationship between the amount of pollen released in wind-pollinated species and fruit (Caccavari *et al.*, 1997, Oteros *et al.*, 2014, Latorre & Belmonte, 2006) and also between pollen and seed production (Latorre & Fassola, 2014). Pollen data can provide information concerning the final harvest several months in advance (García-Mozo, 2011). The relationship between the pollen produced and the harvested seeds observed in this work was direct, supporting the hypothesis proposed. Limitation in seed harvest is associated with the low number of pollen grains that can fertilize the ovules.

According to Anselmini *et al.* (2006), the high temperatures from December to April, during the seed filling period, have a positive effect on their maturity. In SA, there was a low number of seeds per cone, well below the number of seeds produced in 25M, denoting an important difference between sites for this reproductive phenological stage. The maximum temperatures from December to April are lower in SA as well the amount of pollen registered in this site, positively associated with a lower amount of seed production. The above suggests that there is a direct relationship between the number of pollen grains and seed abundance, and also confirms the influence of temperature on this phenological stage. Paraná pine plants growing in tempered regions yield more pollen grains, and therefore more seeds, compared to those living in their subtropical area of origin. Lloret & Kitzberger (2018) suggest that populations close to the limits of species' climatic tolerance could be reservoirs of genothypic variability so as to face extreme climatic events and repopulate disadvantaged sites due to individuals' loss.

Taking into account that global surface temperature is projected to warm constant and progressively (IPCC, 2001-2007), the possibility of displacing the population limit of spatial distribution of *A. angustifolia* to more benign weather conditions for its reproduction, is being considered. The establishment of new populations further to the south of their place of origin, in temperate climates where the environmental conditions favor productivity, maintaining the plantations in Buenos Aires province, would imply a genetic reservoir and seed bank for native forest enrichment or restoration as well as for reforestation in their region of origin (Pinazo *et al.*, 2016), where natural regeneration is minimal. Airborne pollen monitoring data can be considered as a climatic indicator (Fernández-Llamazares et al., 2014).

In situ and *ex situ* conservation units are an effective mechanism to prevent species extinction. *In situ* conservation with seeds from reservoirs or seed banks such as 25M could preserve this species in its natural habitat, providing an important link between the remnants of the native forest. As a parallel and complementary strategy, *ex situ* conservation banks should be established to "safeguard" the genetic variability of the individuals present within the fragments of unprotected areas or close to regions of great demographic pressure (Bittencourt, 2004).

Conclusions

- *A. angustifolia* populations that develop in a temperate climate with cold winters produce a greater amount of pollen that leads to enhanced seed production.
- The current weather conditions encountered in the region of origin of *A. angustifolia* in its southern limit do not favor pollen production, and are affecting the reproduction of this species.
- 25 de Mayo is a suitable location for the establishment of an *ex situ* seed bank.

Since many years of empirical evidence are necessary to validate the conclusions outlined above, pollen production of *A. angustifolia* is continued uninterruptedly along with the quantity of the seeds produced by this species in both regions, and the analysis of the effect of climatic conditions (especially temperature).

This work provides a platform for further investigation on the reproductive phenology of *A. angustifolia,* and allows to establish appropriate management strategies for the maintenance of this critically endangered species.

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