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Comparison between phenological and aerobiological patterns of some arboreal species of Mar del Plata (Argentina)

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Abstract

An atmospheric pollen survey and a periodical quantitative check of the flowering phases of trees were performed during 1993. Most of specific flowering seasons lasted from 4 to 8 weeks. October is the month with the highest number of species in bloom. Anemophylous species flower mainly in coincidence with the relatively low temperatures of late winter and early spring, while the enthomophylous ones correlate with the high temperatures of late spring and summer. The anemophylous 'community' follow a sigmoid pattern with respect to both the flowering development and the cumulative pollen concentration. Agreement between floral phenophases and pollen counts were evaluated for 14 genera (27 species). Pollen data of anemophylous species coincide fairly well with the phenological behaviour. Particular features must be taken into account to interpret this relationship. © 1997 Elsevier Science Ireland Ltd.

Keywords: Aerobiology; Floral phenology; Airborne pollen; Urban trees; Quantitative method; Flowering season

1. Introduction

The flowering of plants in relation to the seasonal climatic changes (floral phenology) is the main factor establishing the time and amount of atmospheric pollen concentration (Jäger et al., 1991). The occurrence of a phenological phase (phenophase) is dependant on both the biotic characteristics of the plant species and the climatic characteristics of the situation in which the species lives (Puppi Branzi and Zanotti, 1992). Each step of the aerobiological pathway (emission, dispersion and deposition or impact) is linked to different biological phenomena that are governed by different meteorological factors. It must be noted also that the meteorological factors may have different effects (Comtois and Sherknies, 1987). This makes the situation very complex. So the shapes of the pollen curves do not always agree with floral development.

The presence of filters, the aerodynamic morphologies of pollen grains, the territorial distribution of pollen sources, are agents that also yield distortions in

the repartition of different pollen types. These modifications could be revealed as a delay between the emission and the capture of grains (Ickovic et al., 1989) or as a lack of coincidence between maximum flowering and maximum pollen concentration. Pollen grains can also be present in air long after the flowering ends (O'Rourke, 1990a).

In addition, pollen morphology seldom leads to the species characterisation owing to the fact that the material is fresh and fixed on the slide. So the systematic analysis by taxa includes a qualitative imprecision (Boussioud-Combières and Barthélémy, 1990). There are also families within which it is difficult to distinguish genera or species under the optical microscope.

It is very important to know blooming periods to correlate with the corresponding aerobiological curves (Zerboni et al., 1986). Flowering calendars are useful but they do not take into account the intensity of the emission (Boussioud-Combières, 1988). The seasonal characterisation of the pollen production is better defined by means of a periodic and quantitative checking of the floral phenophases of the species present in the area (Puppi Branzi and Zanotti, 1992). A quantitative assessment of the characteristics of pollen source activities is extremely important for pollen counts forecast to prevent pollinotic affections (Zerboni et al., 1991). Hay-fever is a disease of which the patient suffers exactly and only at the time when his own allergenic plant is blooming (Geller-Berstein et al., 1991).

The aim of this paper was to analyse the effectiveness of the aerobiological records as an indicator of the phenological events of the emitting flora. To achieve this goal, a method was applied to arboreal species that made it possible to reproduce quantitatively the flowering phenology patterns concerned to the anthesis process. The plant species source in the pollen curve was then identified.

2. Materials and methods

The study was carried out in Mar del Plata (38°03 S, $57°33$ W), Argentina. This city is situated in the southeast of South America, along 10 km of Mar Argentino.

A periodical check of the blooming season of arboreal and shrubby species present in 2.25 km^2 around the pollen sampling site was made. The sampling period lasted from April 1993 to March 1994.

Only the beginning and the end of the blooming season were registered for zoophylous species whose pollen grains seldom are registered in the air (Pérez, 1991; Bianchi, 1994; Latorre and Bianchi, 1996).

Measurements of flowering phenology were carried out twice a week. The phenological survey was made for 25 anemophylous species. There are anemophylous and zoophylous species belonging to a certain genus. In those cases, zoophylous were also checked. For each species a random sample of 5-15 individuals was selected. To follow the anther maturation process, the number of closed, open and fallen flowers (or inflorescence) of 20 branches chosen randomly were counted on each tree, on each day of sampling (Latorre, 1993). In some cases, the anther colour or its stage (immature, emitting pollen, dry) was the characteristic observed. Data were expressed as flowering percentages: number of open flowers with respect to the total by branch and by tree. A branch refers here to the product of the previous year's growing season. When at least one flower of a certain inflorescence was open, it was considered as an 'open flower'; the end of flowering was taken into account when all the anthers were dry.

This phenological technique was difficult to apply to seven species *(Alnus glutinosa, Corylus avellana, Salix alba, Liquidambar sthyraciflua, Quercus* spp. and *Celtis australis)* because of the smallness of their flowers, so the method was slightly modified. For these species a branch was considered in bloom when at least one flower (or inflorescence) from it was emitting pollen.

A scale according to Marcello (1935) was applied to the Myrtaceae species, because the great height of *Eucalyptus* trees made it difficult to examine the flower stages in detail. These plants were checked weekly.

The start of the flowering season of each species was determined as the day when at least one of the selected trees has open flowers and the end when all the flowers had dry anthers.

The more important anemophylous species were considered as a whole to describe the phenological development of the 'anemophylous community'. The twenty-five species were: *Eucalyptus globulus, E. cinerea, E. syredoxylon, E. camaldulensis, Callistemon lanceolatus, Fraxinus excelsior, F. americana, F. excelsior* var. *aurea, F. ornus, Ulmus pumila, U. procera, Alnus glutinosa, Corylus avellana, Salix alba, S. caprea, Acer negundo, Celtis australis, Populus canescens, P. x canadensis, Betula pendula, Platanus acerifolia, Juglans regia, Liquidambar sthyraciflua, Quercus ilex* and Q. *robur.*

A rank from zero (close flowers) to nine (dry anthers), where each class includes 25% of flowering, was constructed. Classes 1, 2, 3 and 4 correspond to the first half of the flowering curve: from 1% of flowering up to the maximum (100% of open flowers). Classes 5, 6, 7 and 8 correspond to declining flowering phases: from 99% of open flowers to 1% of flowering. The flowering pattern fits well by a logistic equation whose form is: $F = K/(1 + e^{(a-rT)})$. Here F means the variable that describe phenophases, while T is time in weeks (since the first one of January until the phenophase observed). The others mean constants; r is related to flowering speed and K is the upper asymptote (empirical model of Puppi Branzi and Zanotti, 1989). To estimate the quantitative relationship between the phenological variable (F) and time (T) , the above equation is transformed in a straight line through the following equivalent equation: $Z_F = rT - a$, where Z_F is the logarithmic function of F (Puppi and Zanotti, 1988). The same criterion was applied to percentages of accumulated pollen concentration of the genera corresponding to anemophylous species assemblages (pollen variable).

Correlation between different flowering variables and temperature was calculated. Meteorological data were obtain with a semi-automatic weather station installed near the pollen trap.

Atmospheric pollen monitoring was performed using a continuous, volumetric and isokinetic sampler (Burkard) located 15 m above ground level. It was placed on a terrace of the University of Mar del Plata building. Twelve hourly trips were analysed each day (Käpylä and Penttinen, 1981). The sampling period was the same as that of the phenological study. Three months of pollen counts (December 1993-February

1994) were lost because of motor failure of the sampler. Daily pollen concentration of each pollen type was calculated using a modified formula of O'Rourke (1990b) and expressed as number of grains per cubic meter of air.

3. Results

The flowering season of 80 species was analysed. They represent 51% of the specific diversity in the area. Most of their blooming season extended from 4 to 8 weeks. Eighty per cent of anemophylous species (in short 35) have allergenic potentiality, while only 13% of zoophylous species have this characteristic (Table 1).

The arboreal species considered as a whole ('community') showed a seasonal pattern of blooming along the year (Fig. 1). A weekly mean of two species has open flowers during summer and autumn. The number of species in bloom starts to increase in winter. During September and October, weekly mean of the number of flowering species were 7 and 10 respectively. The maximum was detected in October, with a peak of 30 species flowering in the third week.

Most of the anemophylous species flower during late winter-early spring, in coincidence with the highest pollen diversity. Zoophylous species flower from late

Fig. 1. Weekly pollen diversity, number of zoophylous and anemophylous species in bloom and weekly mean temperatures in Mar del Plata from May 1993 to April 1994.

Fig. 2. Floral phenological development of the arboreal anemopbylous 'community' of Mar del Plata city and the corresponding cumulative pollen concentrations in 1993.

spring and most of them are on anthesis in summer while the anemophylous have finished the last floral phenophase. There is a significant negative correlation between weekly means of temperature and the number of anemophylous species flowering during the year $(r =$ -0.34 , $P < 0.0001$). On the contrary, the correlation is positive with the zoophylous species $(r= 0.42, P <$ 0.0001) (Fig. 1).

Floral phenological changes of the 'anemophylous community' seem to fit a sigmoid pattern (Fig. 2) that may be approximated by a logistic equation. The resulting regression equation was: $Z_F = 0.355T - 12.488$, with $P < 0.0001$, $r = 0.996$ and $R^2 = 99.14\%$. The weekly correlation coefficient between phenophases and means of temperature was 0.906 ($P < 0.0001$).

Percentages of accumulated pollen assemblage also seem to follow a sigmoid pattern (Fig. 2). The linear relationship between pollen variable (P) and time (T) is highly significant ($P < 0.0001$) resulting in the following regression equation: $Z_p = 0.399x - 12.960$, with $r =$ 0.975 and $R^2 = 95.05\%$.

On average, pollen grains of the anemophylous species were registered 5 days before the flowering season starts (as defined above) and they remain in the atmosphere 17 days after the flowering ends.

3.1. Results on flowering and pollination of main species (Fig. 3)

3. I.I. Fraxinus

This genus is represented by four species, two of winter pollination *(F. excelsior* and *F. americana)* and two *(F. excelsior* var. *aurea* and *F. ornus)* of spring pollination. *F. excelsior* starts to bloom on 30 June; its first phenophase develops very slowly and the last

Table 1

Type of flowering of arboreal species: brief (upto 4 weeks), medium (4-8 weeks) and extended (more than 8 weeks)

Table 1 (continued)

Anemophyllous and allergenic species are indicated with an asterisk. Abundance expressed the number of trees in 2.25 km^2 .

phenophase superimposes with the initial phenophase of *F. americana.* **Forty-seven days after that,** *F. americana* **ends its flowering while the spring-blooming species begin to open their flowers.** *F. ornus* **flowering season spans till the last days of December, with two peaks of flowering occurring.**

Three principal peaks of high concentration were observed in the atmospheric pollen curve of *Fraxinus.* **The first one coincides with the** *F. excelsior* **flowering period and the second one with** *F. americana.* **This is confirmed by the optical determination of grain morphology. Twenty-three per cent of** *F. americana* **pollen**

Fig. 3.

Fig. 3.

Fig. 3. Floral phenological pattern of each species and aerobiological curve of the corresponding pollen type during 1993 in Mar del Plata.

concentration was registered after its blooming season had finished. After these two peaks, it seems that a pollen assemblage of those species that flower later exists. It is noticeable that the scarce amount of F. *ornus* pollen obtained does not correspond with its phenological stage.

3.1.2. Quercus

There are two main species in the area: *Q. robur* and *Q. ilex,* a few trees of *Q. palustris* are also present (Table 1). The flowering season of *Q. robur* lasts from the middle of September to the middle of October. Ten days later, *Q. ilex* flowers until the end of November. Floral phenophases develop very fast in both species. Male flowers are functional during a short time of about 1 month.

The *Quercus* pollen curve falls in with the *Q. robur* flowering curve. It can be observed that a certain amount of grains registered before the *Q. robur* flowering season ends. This remaining pollen shows a peak in coincidence with the *Q. itex* floral phases. In agreement with the microscopic determination of grains, remaining pollen can be assigned to evergreen species (170 grains/ $m³$).

3.1.3. Ulmus

U. pumila is the principal species planted along streets. It starts to flower on 3 July and ends by early September. The progress of initial phenophases is slow. The *U. procera* flowering season overlaps with that of *U. pumila,* but it is more brief. Both flowering peaks coincide around 20 August.

The pollen curve seems to fit with *U. pumila* floral development.

3.1.4. Acer

Three species are present along the streets. The more abundant is *A. negundo* that flowers on 26 August and finishes on 12 October. *A. campestris* and *A. pseudoplatanus* start to bloom in the middle of September but the first one ends its flowering by the time *A. negundo* does, and the second 1 month later.

Acer pollen concentration curve coincides mainly with the *A. negundo* flowering patterns. The pollen peak is registered exactly when the maximum flowering of that species occurs.

3.1.5. Salix

The two species in the area are *S. eaprea* and *S. alba.*

They are in bloom from the middle of August to the middle of October. Initial phenophases of *S. caprea* are fast while in *S. alba* the final stages are faster.

The beginning of pollen detection occurs on 24 August. Pollen remains in the air some days after the end of flowering of the both species. High concentrations are registered when initial floral phenophases of both species are developing.

3. I. 6. Myrtaceae

The flowering season of *Eucalyptus* and *Callistemon* species are registered from May to December. C. *lanceolatus* is the species commonly planted in gardens and streets and blooms twice during this period. The *E. globulus* flowering season lasts for 7 months with an intermittent floral rhythm. A high variability between branches within a tree of this species was observed. Its density is very high compared with the other species of *Eucalyptus.* The *E. cinerea* flowering season is also long; *E. siredoxylon* and *E. camaldulensis* have a blooming period comparatively shorter (4 months).

Myrtaceae pollen is collected throughout the year. The occurrence curve is rather irregular as may be expected for a multi-species assemblage. The two main peaks of high concentration (June and September) adjust with the blooming of two complexes integrated by: *E. globulus -- E. cinerea -- C. lanceolatus* and E . globulus $- E$. camaldulensis $- E$. siredoxilon.

3.1.7. Alnus

Alnus pollen grains were registered in a low concentration and only on 2 days (6 August and 12 September). It was coincident with the start (10/8) and finish (9/22) of the flowering season of *A. glutinosa.*

3.1.8. Betula, Liquidambar, Platanus and Corylus

These genera are represented mostly by one species of vegetation in the study area. *C. avellana* shows a winter blooming (in August), while *B. pendula, L. styracifhlua* and *P. acerifolia* flower in spring during a short time span.

A common feature joins these species: each phenological stage agrees with a pollen concentration change. Start, beginning and end of flowering correspond with the pollen curve.

3.1.9. Juglans, Celtis and Populus

The flowering period of *C. australis* is short (September). This is the unique species in the study area. *J. regia* and *Populus* (mainly *P. x canadensis* and P. *canescens)* floral seasons occur during September and October.

Pollen curves of these types do not agree at all with the phenological development.

Flowering and pollen maximums of *Celtis* and *Juglans* are coincident, but initial floral phenophases are slow while pollen concentrations increase in a short time. On the contrary, the final stages of flowering develop quickly while pollen concentrations decrease slowly for both taxa.

Pollen grains of *Celtis* and *Populus* remain in the atmosphere while the flowering seasons have ended. This is very noticeable in *Celtis* curves.

Phenological variability between trees is detected in those species where flowering percentages do not reach 100%. This is noticeable in *Fraxinus* spp., in S. *caprea* and in *L. stiracifhua* (Fig. 3).

4. Discussion

Most of the woody species that grow in Mar del Plata have a spring flowering season, which extends between 4 and 8 weeks. Similar results were found in other cities of Buenos Aires Province and the Mesopotamia Region of Argentina (Mathov, 1972) and in cities of Chile (Aljaro and Hoffmann, 1979). A unique flowering pattern was that of *Eucalyptus,* with a long period of about 10 weeks.

Opler et al. (1980) classified reproductive behaviour in three dichotomous categories: continuous/discontinuous, brief/extended and synchronous/asynchronous. The continuous pattern of flowering is represented in Mar del Plata by the majority of tree species and the discontinuous one by *Callistemon;* the brief pattern by *Platanus* or *Populus* and the extended by *Eucalyptus;* the synchronous by *Populus* spp. or *Ulmus* spp. and the asynchronous by *Fraxinus* spp.

From late winter, anemophylous species start to bloom with a peak of flowering species in spring. In November, these species finish their blooming seasons by the time the enthomophylous start to open their flowers. There are few species with an autumnal flowering.

The marked floral seasonality of anemophylous and enthomophylous taxa is related with the climatic seasons of this region. Anemophylous flowering relates with the low temperatures of winter and early spring, while zoophylous flowering would be influenced by high temperatures of late spring and summer (Smith-Ramirez and Armesto, 1994).

The anemophylous community shows a progressive phenological pattern through the time that lasts from July to November. First floral phenophases develop very slowly, followed by faster changes until the final phenophases with a slow velocity of flowering (Fig. 3). This pattern is associated with the progressive increase

of temperatures. July was the coldest month of the period; then, there is a gradually increase in temperature. The relationship between phenology of anemophylous species and temperature is proven by the highly significant correlation between floral phenophases and weekly means of temperatures.

The accumulated pollen curve follows the same pattern as those of floral development. That is indicative that aerobiological data fit well into the phenological curve. Final stages of accumulated pollen are quicker than those of phenological progress.

To evaluate the aerobiological survey as indicative of flowering stage, the zoophylous taxa were not included. The presence of these pollen grains in the atmosphere is not always related to the corresponding phenological phase of the pollen source (Latorre and Bianchi, 1996). On the contrary, the atmospheric pollen register precisely indicates the start and maximum of the anemophylous flowering phase. Only the end of the blooming season is not well indicated, thus pollen grains remain in the air long after the anthers fall (O'Rourke, 1990a). The sequence of temporal concentrations can be reviewed as a derivation of a production exposed to proportional losses. This leads to a log-normal distribution (Janssen, 1973) where the onset of pollination is sudden (and more predictable) and its decline gradual (Comtois and Sherknies, 1987). This difference in final stages can be annulled if we consider the principal pollination period determined *sensu* Nilsson and Persson (1981). The flowering finishes in coincidence with the date when 95% of pollen was detected. Also, the start of flowering is before this and precedes by 5 days 5% of accumulated pollen concentration.

4.1. Discussion on flowering and pollination of the most *important species*

Most of *Fraxinus* grains come from *F. excelsior* and *F. americana* pollen sources (80% of genus concentration). The pollination period of *F. excelsior* corresponds exactly with its flowering season. On the contrary, a great amount of *F. americana* pollen remains in suspension after its blossoming season ends. There could exist a combined effect between this pollen type and the pollen from *F. excelsior* var. *aurea.* The greater number of *F. americana* plants and its high intraspecific floral phenology variability (indicated by the low flowering percentages reached) result in the high pollen concentration observed and its consequent maintenance in the air. The other pollen source is *F. ornus* whose pollen grains are almost not registered. This would be caused by the scarce number of trees. Additionally, the sterile parts of flowers and the presence of leaves during its pollination period could act as a filter limiting pollen shedding into the atmosphere.

The morphological determination of *Quercus* pollen grains *(Q. robur* and *Q. ilex)* is supported by the coincidence of the specific flowering curve and that of pollen. Due to the relatively low density of *Q. ilex* trees, the concentration registered is very low. In addition, *Q. ilex* is an evergreen species whose leaves can act as a filter to pollen emission, like in the previous example and in contrast to Q. *robur* morphology. Other causes of the scarcity of Q. *ilex* are the distribution of plants and the wind prevalence. This species is arranged in groups as little artificial forests located west from the pollen sampling site. The prevailing winds during its blossoming period came from the south -- south-east and the north -- north-east directions.

The flowering and pollen season of *Ulmus* last for the same period and the shape of both curves are nearly isomorphic. Maximum pollen concentration is reached when *U. pumila* and *U. procera* are flowering.

The peaks of open flowers of *Acer pseudoplatanus* and *A. campestris* are simultaneous but they are not important sources with respect to the pollen influx. According to field observations, these two species are visited by insects; besides their flower morphology is related to entomophylous pollination. *A. negundo,* which flowers earlier, is the principal pollen source. The morphological structure of the flowers is adapted to the anemophylous pollination and supports the previous conclusion.

Maximum *Salix* pollen concentration occurs at the start of the flowering of *S. alba* and *S. caprea. Salix* is considered an entomophylous genus by some authors or with anemophylous features, by others. The entomophily can be attributed, especially for S. *caprea,* which is continuously visited by bees during its pollination period and also its pollen grains are sticky. In addition, the low density of trees carries a low concentration of pollen grains into the atmosphere. Despite this, no time lag was observed between flowering and pollen in the air. The high amount of pollen emitted into the air from these plants (Faegri and van der Pijl, 1979) can be the reason of the simultaneous nature of both phenomena.

From May to December pollen of the Myrtaceae was registered by the time that *Eucalyptus globulus* was in its flowering season. This species and those of the *Callistemon* species, are dominant within this family in vegetation. Myrtaceae airborne pollen curve shows variations in concentration throughout the year that would correspond with successively flowering periods of different species; there is a superposition of floral phenophases. Taxa grouping large families show a diverse set of frequency towards flatness and multimodality. The *Eucalyptus* blooming season is long and that of *Callistemon* is discontinuous.

Few *Alnus* pollen grains were registered but they were caught by the time when flowering was occurring. There are few trees that can be the source of this pollen, and they are located 700 m distant from the sampling site. Previous studies shown that this pollen type is generally over-represented when the plant source is close to the sampler. During 1990, with the same sampler in the same place, *Alnus* pollen reached high concentration. These grains came from an individual plant that was located in the University garden which has since died (Latorre and Bianchi, 1996).

The flowering and the pollen registered were coincident in their principal phenophases (start, maximum and finishing) except for *Betula, Liquidambar, Platanus* and *Corylus.*

The first pollen peak of *Celtis* corresponds to the *C*. *australis* blooming season. This species is the only one found in Mar del Plata. Long after *C. australis* had ceased flowering, a relatively high frequency of *Celtis* pollen was found which may be from *C. tala.* This native species is located decades of kilometres to the north and northwest and flowers later than *C. australis.* Thus, the abundant pollen during October and November can be considered as a regional effect; there seems no other plausible explanation.

Except for the Myrtaceae species, *Cryptomeriajaponica, Casuarina cuninghamiana* and *Nothofagus* sp., all the anemophylous tree species have an allergenic potentiality. This is caused by the great amount of pollen grains in the atmosphere that can produce pollinotic effects on the human respiratory tract (Famularo et al., 1992). This is important for the forestry planning of a city and the adoption of pruning measures to avoid harmful effects.

Some pollen grains of the enthomophylous tree species can be allergenic like *Tilia cordata* and *Prunus* spp. (Negrini, 1992). These two genera are very common in the city but their pollen concentration is nil and very low, respectively. In these cases, the interest is limited to the proximity between the sensible individual and the pollen source.

5. Conclusion

A general conclusion is that aerobiological analysis makes it possible to conduct a detailed flowering survey. Variations in pollen concentration correspond with different floral phenophases. Pollen concentrations in the air are dependent on the innate pollen release rhythm of plant species.

The complexity of reproductive strategies and its expression in the airborne pollen spectrum, make it necessary to incorporate phenological variables to study aerobiota (Solomon and Durham, 1967). The analysis of phenological data makes it possible to access the flowering periods and the specific pollen peaks to determine what species is responsible (Zerboni and Manfredi, 1989). Phenological data are the base, and in some cases the only empirical support to interpret atmospheric pollen data (Zerboni and Manfredi, 1988).

It was observed and described that the exact flowering period of allergenic plants correlated fairly well with the time course of their pollen counts (Keynan et al., 1989). Airborne pollen pattern of each genus seems to fit with the flowering patterns of the emitting flora. Since the climatic features of the station, and consequently phenological behaviours vary from year-toyear, it is also necessary to consider the variable year.

To describe this behaviour it would be necessary to follow phenological observations during some years because of the climatic variations influence on flowering periods of many species (Zerboni et al., 1991). So, further phenological and aerobiological sampling are been made now. Whereas, small fluctuations could exist at the start and finish, the long duration of the flowering season is constant for each species (Aljaro and Hoffmann, 1979). The dispersal pattern is fixed in the population biology of the species and repeats itself year after year (Comtois and Sherknies, 1987). In addition, the weather will be analysed in futures studies to determine its influence on each phase of the aerobiological pathway.

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