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Fabiana Latorre^a & María Martha Bianchi^b

^a Lab. Palinología, Dpto. Biología, FCEyN, Universidad Nacional de Mar del Plata, Funes, Mar del Plata, 3250 (7600) E-mail: latorre@mdp.edu.ar

^b PROGEBA-CONICET, Apartado postal 47, S. C. de Bariloche, (8400), Argentina

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Relationships between flowering development of *Ulmus pumila* and *Fraxinus excelsior* and their airborne pollen

FABIANA LATORRE and MARÍA MARTHA BIANCHI

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The flowering patterns of *Ulmus pumila* and *Fraxinus excelsior* were studied during the 1990 growing season in order to investigate their pollen dispersion curves. The use of airborne pollen records as predictors of flowering of allergenic trees was evaluated. A sampling method to describe quantitative flowering phenophases was applied. A Burkard trap recorded airborne pollen in the city of Mar del Plata. The phenological sampling method showed that floral phenophase development is not always synchronous between trees. The variability registered was larger for *F. excelsior* than for *U. pumila*. A delay between flowering and the airborne pollen recorded was not noticed for *Ulmus*. The greatest amount of *Ulmus* pollen came from the local vegetation, mainly from *U. pumila*. *Fraxinus* pollen was recorded for a longer period after the *F. excelsior* flowering season the ended. *Fraxinus* pollen income came from *F. excelsior* but also from *F. americana* and *F. excelsior* var. *aurea* that flower later. An immediate decrease in airborne concentration was noticed for both pollen types following rain. When relatively strong rainfall was registered a decrease in the number of open flowers occurred only in *F. excelsior*.

Fabiana Latorre, Lab. Palinología, Dpto. Biología, FCEyN, Universidad Nacional de Mar del Plata, Funes 3250, (7600) Mar del Plata (e-mail address: latorre@mdp.edu.ar); María Martha Bianchi, PROGEBA–CONICET, Apartado postal 47, (8400) S. C. de Bariloche, Argentina.

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Airborne pollen studies contribute to the understanding of plant community dynamics by means of recording changes in the phenological features of the vegetation (Fournier 1974, Schnelle & Volkest 1974, Taylor 1974). Although pollen emission and pollen concentration in the atmosphere are different phenomena, they are integrated in the aerobiological pathway as both are influenced by the interaction of endogenous (phenological) and exogenous (environmental) factors (Subba Reddi & Reddi 1985).

Aerobiological studies must be supported by field studies in order to determine to what extent airborne pollen concentrations represent different floral phenophases (Agashe & Alfadil 1989, Puppi Branzi & Zanotti 1989) and quantitative phenological studies are necessary to identify and characterize variations in floral phenophases between individuals and among species. These studies are most important when calculating pollen production (emission) rates and pollen concentration curves. Although quantitative data are important, most of the studies show a qualitative approach and standard criteria to quantify flowering phenophases are still lacking (Jackson 1966, Augspurger 1981).

In this work we analyzed the relationships between the reproductive phenology patterns of urban trees and their airborne pollen curves to study the distortions between the release of pollen from the anthers and its presence in the atmosphere. For this purpose, a quantitative sampling method for determining the floral phenophase pattern of urban trees was applied. Two arboreal species, allergenic pollen producers: *Ulmus pumila* and *Fraxinus excelsior*, were chosen for this study.

MATERIAL AND METHODS

Environmental characteristics

The climate of Mar del Plata, Argentina (Lat. 38°03'S Long. 57°33'O) is mesothermal, sub-humid-humid without water stress.

The city is situated along 12km of Mar Argentino on flat, low hills. Crops (wheat, maize), weeds and indigenous grasses characterize the regional vegetation. Exotic trees such as *Ulmus*, *Fraxinus*, *Platanus*, *Populus*, *Acer*, *Prunus* etc. grow in the parks and streets of the city. A residential quarter near the sampling site has abundant trees of the taxa *Quercus*, *Cupressus*, *Betula* and *Eucalyptus* (Latorre & Bianchi 1997). The area selected for this study is representative of the whole city, except for downtown where sparse vegetation exists.

Following Solomon & Hayes (1980) a census of urban arboreal vegetation was carried out for an area of 1 km² centred on the pollen sampler, in order to register the principal pollen sources (local and extra-local plants; Prentice 1985).

Phenological survey

We choose the plant species for inclusion in this analysis according to five criteria. 1) To be representative of urban vegetation; 2) to be registered in the airborne pollen calendar of the city; 3) to have allergenic significance; 4) to be wind pollinated; 5) to have overlapping flowering periods (to make comparisons independent of any climatic influence). Two species were selected on the above grounds: *Ulmus pumila* (elm) and *Fraxinus excelsior* (ash).

To characterize floral phenopases quantitatively, a sampling method was applied during the whole flowering period (from July to August 1990).

Selection of individuals. – A sample of 9–10 individuals chosen randomly within the study area was used for each species (Fournier

et al. 1995, Opler et al. 1980). The number of chosen trees represented 1.5% of the total elms and 50% of the total elm and ashes growing within the sampling area. To avoid bias of capture due to the variable wind direction prevailing in the city, we chose trees located at different orientations. All of them were located in sunny places.

To evaluate the effect of pruning on the flowering of *Ulmus pumila* five trees pruned the previous year and five un-pruned trees were sampled.

Periodicity of sampling. – Counting was carried out twice a week. The same plants were monitored during each sampling session.

Counting technique. – Twenty branches of the selected trees were chosen randomly. They were different each day of sampling and taken from all parts of the crown to avoid local microclimate bias (sunny or shade parts, base or top). Only the segment grown since the previous flowering period was considered.

For *U. pumila* the number of flower buds (closed flowers), open flowers (exposed anthers) and non-functional flowers (without stamens and petals) were computed on each branch.

For *F. excelsior*, the number of closed inflorescence (red anthers), open inflorescence (yellow anthers with pollen) and non functional inflorescence (dry and black senescence anthers) were considered. The average number of open flowers/inflorescence per branch and per tree was used for the analysis.

Airborne pollen sampling

A continuous, volumetric sampling of atmospheric pollen was performed. A seven-day volumetric pollen trap (Burkard) located 15 meters above ground level was used. The sampling period was six months long, from June to November, which is the flowering period of most of the local tree species.

The method of slide preparation for microscope analysis has been described by Bianchi (1994). Slides were examined at 200× magnification, following transverse traverses (Käpylä & Penttinen 1981). The daily pollen concentration was calculated after O'Rourke's model (O'Rourke 1990).

Data analysis

A t-test was applied to the *U. pumila* data to compare pruned and unpruned trees. One-way analysis of the variance (ANOVA) and Scheffé test (a posteriori) were carried out to analyze the differences in floral development within and between trees of each species. The significance level was established at 0.05. A variation coefficient of flowering percentages was calculated. A Spearman rank correlation was calculated to analyze flowering and pollen correspondence (Winer 1971, Sokal & Rohlf 1979).

The principal pollen season for *Ulmus* was determined following the methods of Nilsson & Persson (1981) and Mullenders et al. (1972). Both methods are usually applied to any kind of pollen type. Nilsson & Persson (1981) defined the main pollen season as the period from which the sum of concentrations reaches 5% of the total sum until the time when the sum reaches 95%. Mullenders et al. (1972) defined the main period beginning on the day when the concentration of five days reaches at least 1% of the accumulated total during three consecutive days and end when the concentration is less than 0.9% during more than 10 days.

U. pumila and *F. excelsior* principal flowering seasons were determined with the same criterion that Nilsson & Persson (1981) applied to pollen data.

The Servicio Meteorológico Nacional collected the rainfall data. These data were used to analyze the effect of rain in the pollen-flowering relationship.

RESULTS

Ulmus

Flowering season of *U. pumila*. – Although the pruned plants flowered with a time lag of 4 days, the mean flowering percentages of the pruned and un-pruned trees were, in all cases, not significantly different (Student $-1.82 \leq t \leq 0.47$; $0.107 \leq p \leq 0.793$). Therefore, all individuals were considered as the same statistical population for further analysis.

Differences between trees were greater than differences within trees, flowering means were always significantly different between individuals ($4.07 \leq F \leq 9.66$ $p < 0.0001$). When we compared all of the possible pairs of trees (Scheffé test), individuals showing major differences at a sampling session were not the same during the sampling period.

For *Ulmus pumila* the flowering season began at the end of July and lasted for a month, with a peak on 10th August. The floral phase between start and peak was twice as long as the decreasing floral phase (between peak and end). During the principal flowering season, which lasted 18 days, 75% of the total flowering was registered (Fig. 1).

Pollen season. – *Ulmus* pollen was registered from July to October, with the maximum concentration registered on

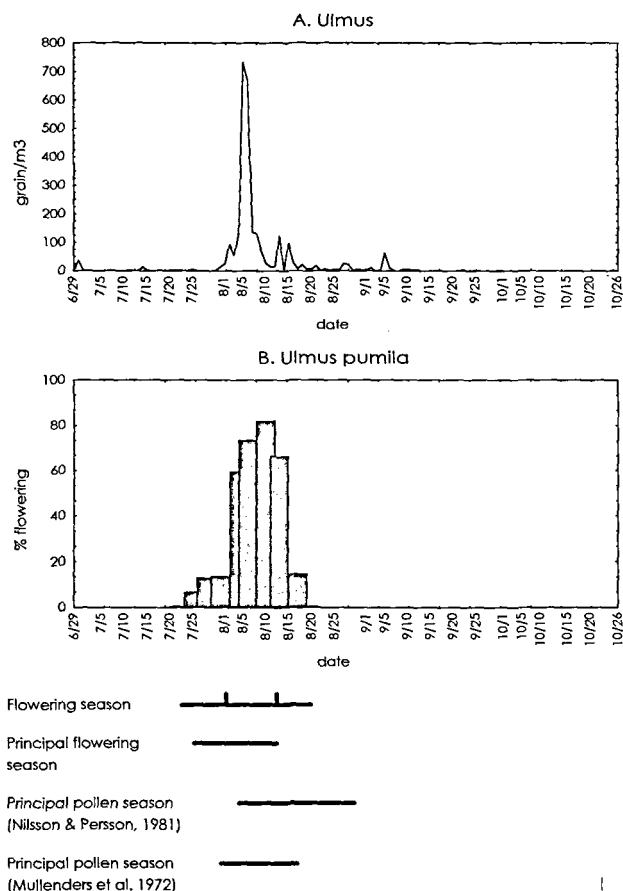


Fig. 1. *Ulmus* pollen concentration (A) and mean flowering percentages of *U. pumila* (B) during 1990 in Mar del Plata. The flowering season and the extent of the principal pollen and flowering seasons are indicated.

6th August (Fig. 1). The principal pollen season (sensu Nilsson & Persson 1981) lasted 25 days, 9 days longer than the flowering period. The principal pollen phase calculated according to Mullenders et al. (1972) lasted only 16 days and was coincident with the maximum peak and with the ending of flowering (Fig. 2). Estimation of pollen concentration was 86.41% after Nilsson & Persson (1981) and 86.40% after Mullenders et al. (1972).

Pollen-flowering relationship. – *Ulmus* pollen was recorded 22 days before the *Ulmus pumila* flowering period started and 67 days after flowering ended. However, only 11.57% of the total pollen count corresponded to the tail of the pollen curve. During the beginning of August both phenomena increased but while the pollen concentration curve decreased sharply, the flowering curve reached its peak (Fig. 1).

A significant correlation between flowering stages and pollen in the air was registered ($R_s=0.78$, $p=0.0076$). Cumulative flowering percentages and atmospheric pollen concentration showed a similar pattern of development (Fig. 2).

Fraxinus

Flowering season of *F. excelsior*. – A high variability of flowering among individual curves was found. It was observed that although the apical inflorescence opened before the basal ones, anther shedding did not follow a continuous pattern. Apparently, a time lag exists between the opening of the apical and distal flowers. The same feature was observed in *Fraxinus ornus*. In this species two flowering peaks two weeks apart were registered.

According to one way ANOVA, mean flowering percentages were significantly different between individuals on each day of the survey ($3.69 \leq F \leq 38.26$, $p < 0.008$). The Scheffé test proved that there are always two or three groups of trees that differ significantly in their flowering mean. These groups did not have the same tree component each day. The variation coefficient of flowering was 116.88%.

The flowering season of *Fraxinus excelsior* was 34 days long and its principal phase lasted only 21 days (Fig. 3).

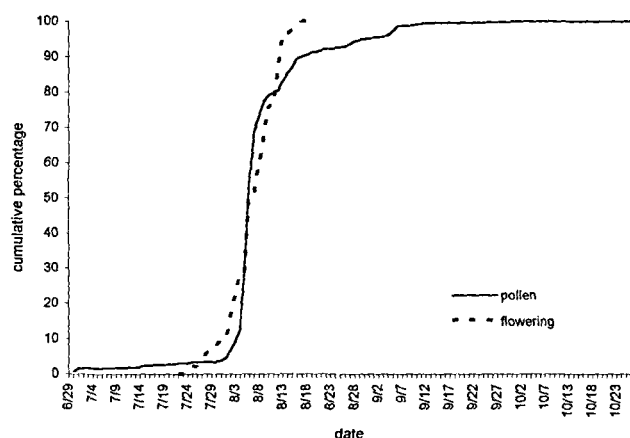


Fig. 2. Cumulative percentages of *Ulmus* pollen and *U. pumila* flowering throughout the 1990 growing season.

Pollen season. – The pollen concentration curve of *Fraxinus* was bimodal, the first peak occurring during July-August and the second one during September-October. Secondary peaks were registered throughout the whole period (Fig. 3).

Pollen-flowering relationship. – The flowering of *F. excelsior* was coincident with the first peak of the pollen concentration curve of *Fraxinus*. 17% of the *Fraxinus* pollen was recorded before the start of *Fraxinus excelsior* flowering. Maximum pollen concentrations were registered during the second peak, before the flowering season ended (Fig. 3).

The rank correlation coefficient between flowering percentages and pollen counts was not significantly different ($R_s=0.20$, $p=0.54$). The slope of the flowering cumulative percentage curve is steeper than that of the pollen cumulative percentage curve. Apparently flowering takes a few days but *Fraxinus* pollen remains in the atmosphere a longer period (Fig. 4).

Rain

Two periods of rain that lasted 5 and 8 days respectively took place during the sampling period. Up to twelve millimeters of precipitation were recorded during the second rainy period (Fig. 5).

Ulmus pollen concentration diminished immediately with the rain even though the *U. pumila* flowering curve had its maximum value.

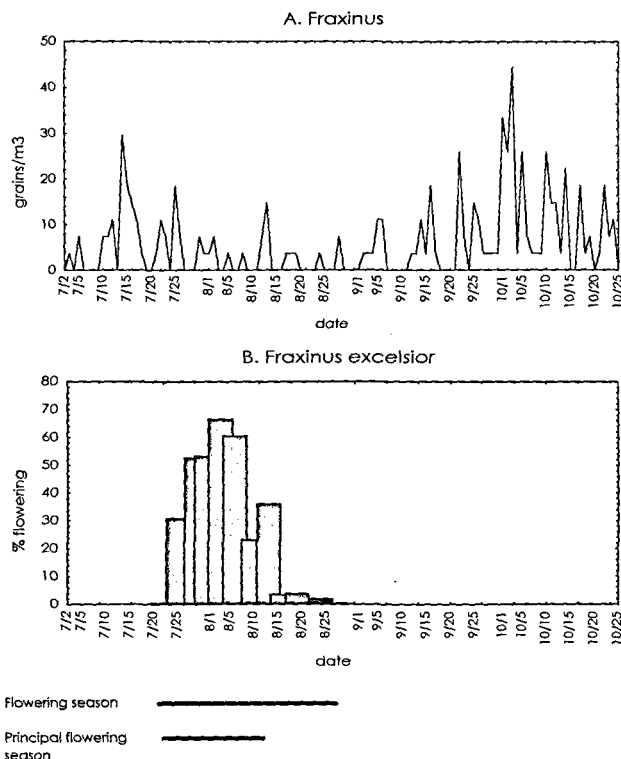


Fig. 3. *Fraxinus* pollen concentrations (A) and mean flowering percentages of *F. excelsior* (B) during 1990 in Mar del Plata. The flowering season of *F. excelsior* and its principal flowering season are indicated.

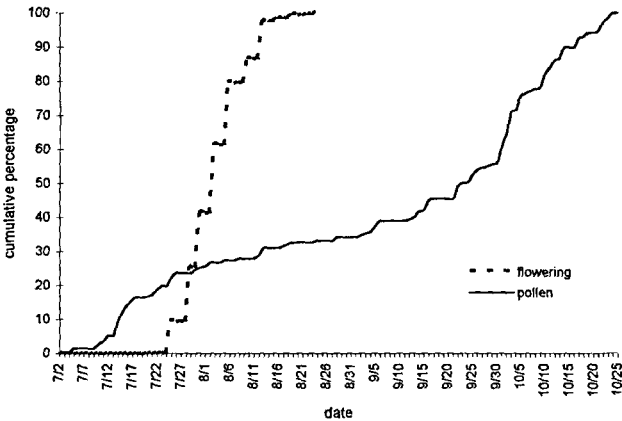


Fig. 4. Cumulative percentages of *Fraxinus* pollen concentration and *F. excelsior* flowering in 1990.

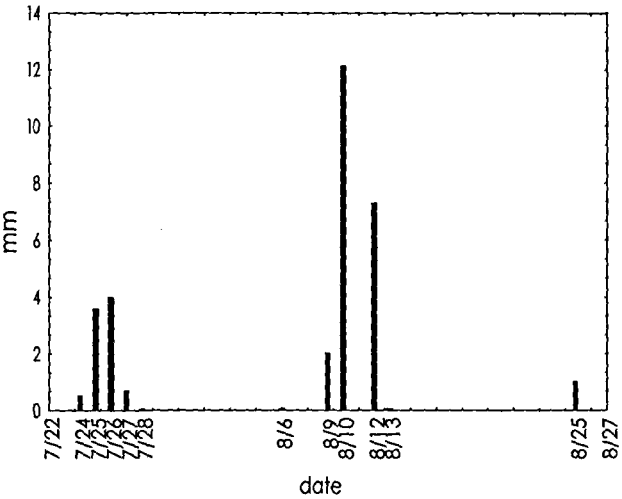


Fig. 5. Precipitation in Mar del Plata in 1990 during the flowering seasons of *U. pumila* and *F. excelsior*.

The first rainy period during the *F. excelsior* flowering season was coincident with the start of flowering and seems not to affect the pollen concentration. A small peak of concentration was registered in these days and diminished after a time lag. Nevertheless during the second rainy period, a higher amount of precipitation was registered. As a consequence, a depression phase in the flowers opening curve is observed. The atmospheric pollen concentration was nill during this period.

DISCUSSION

Ulmus

The observations show that pruning in the previous year had no significant in the flowering pattern of *U. pumila*. All trees showed the same pattern of floral development although momentary differences among trees were observed. Only one principal peak of *Ulmus* flowering was registered.

Ulmus pollen was registered in the atmosphere of Mar del

Plata during a 4 month long period (late winter–early spring). Following the method of Mullenders et al. (1972), the principal pollen period could be defined within the flowering season. Grains recorded outside of this principal pollen phase were due to refloatation phenomena. The refloatated pollen grains showed an absence of cytoplasm and had damaged exines (O'Rourke 1990).

Most of the *Ulmus* pollen recorded came from the local vegetation. The principal pollen phase occurred during the flowering period of some *U. pumila* trees growing next to the pollen sampler. Although pollen from extra-local sources could have been registered, the spatial pattern of deposition (leptokurtic curve shape) and the weak aerodynamic features of *Ulmus* pollen grain suggested a mainly local source for the pollen registered (Janssen 1973, Comtois & Sherknies 1987).

It could be consider that most of the *Ulmus* pollen came from *U. procera* flowering simultaneously with *U. pumila* (Latorre 1997), but its contribution to the atmospheric record would be masked by the abundant pollen concentration of *U. pumila* due to a higher plant density in the area (Latorre & Bianchi 1997).

No delay between the start of flowering and the presence of atmospheric pollen was registered for *Ulmus*. The greater concentration was registered before the maximum flowering.

Rain seems to “wash” *Ulmus* pollen grains from the atmosphere. The pollen concentration decreases sharply in relation to a heavy rain fallen. This fact explains why the minimum pollen concentration was registered when almost all the flowers were open. Richard (1985) found similar results. Rain could have also affected the pattern of flowering during the final stage.

Pruning did not influence the floral phenology development of *U. pumila* trees.

Fraxinus

The progress of flower opening is not uniform either within or between individuals. Variance within the same species could be explained by environmental heterogeneity (Grime 1979). Patterns of flower development could be different in each individual due to different microenvironment conditions. The survey area was not enough large to demonstrate this phenomenon.

The branches of each tree could be interpreted as modular organisms whose flowerings have a different timing and speed of development (Hill & Halverson 1984, Delph 1993). Our data in no way prove that there is a genetic basis to the observed differences in flowering between branches of the same tree. They simply show that in respect to the reproductive characters, the variations observed within a single individual tree were as large as variations registered among different trees.

The fluctuation of pollen concentration in the atmosphere is dependent on the innate pollen release rhythm of the species (Comtois & Sherknies 1987). The dynamics of floral opening and the variability on the rate of flower development could be an adaptation of anemophilous pollination to dioecy present in *F. excelsior* (Tapper 1996).

These different patterns of flowering are reflected in the

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pollen curve with numerous peaks during the whole season. The requirement of a constant presence of pollen to secure ovule fertilization could be favoured by some aerodynamic characteristics of the pollen grains such as weight and moisture, that facilitate their persistence in the atmosphere after release (Aljaro & Hoffmann 1979).

Sometimes, in some families or genera, it is difficult to differentiate species under the optical microscope, especially when the grains are not acetolyzed. Knowledge of the seasons of pollination of species of the same genus helps to solve these limitations.

The long duration of the *Fraxinus* pollen season represents the flowering patterns of the different species cultivated in the city of Mar del Plata. The first peak of atmospheric pollen was related to the flowering of *F. excelsior* while subsequent peaks were associated with species that flower later. The main source for the second peak (including maximum value) was *F. americana* which started to flower after *F. excelsior*. *F. americana* has a higher plant density but a little amount of pollen can be attributed to *F. excelsior* var. *purea* which flowers at the same time as *F. americana* (Latorre 1997). Bimodal patterns of pollen dispersion are also found in *Betula* (Käpylä 1984), *Quercus* (Donini & Sutra 1987, Latorre & Pérez 1997) and *Eucalyptus* (Bianchi 1994).

CONCLUSIONS

The phenological technique employed allowed us to quantify the evolution of flowering of *U. pumila* and *F. excelsior* in the city of Mar del Plata.

Aerobiological methodology proved to be an accurate indicator of the flower phenology of *U. pumila*.

In the case of *Fraxinus*, the high variability found among urban trees suggests that a larger sample would be necessary to evaluate the flowering development of the genus in Mar del Plata. Besides, it would be important to include all possible sources of the species to correlate all *Fraxinus* pollen curves.

A better characterization of atmospheric pollen curves was obtained by means of periodical phenological checking of the flora within the sampling area. The study of periods and timing of flowering can contribute to the prevention of pollinosis, and can also contribute to a better understanding of the phenology of some urban trees.

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