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Phenology and pollen production of *Parietaria judaica* L. in Catalonia (NE Spain)

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Variability of phenology and pollen production of three geographically distant (20–60 km) populations of *Parietaria judaica* L. in Catalonia (Spain) was analysed together with their relationships to airborne pollen dynamic curves. *Parietaria judaica* L. is a perennial herb, with individual plants consisting of many shoots emerging from a common rootstock, most of them flowering copiously in Spring. Flowers are hermaphrodite or female, and pollination is anemophilous. From November 1999 to October 2000 pollen production was estimated from periodical records in three permanent plots per zone. Moreover, records of airborne Urticaceae pollen were obtained daily by means of Hirst spore traps operating in each zone.

Parietaria judaica grows vegetatively and reproductively throughout the year, but intensity of these phenological phases peaks in Spring. The number of pollen grains per anther range from 35 744 to 86 333, and average pollen production is $2\,350 \times 10^6$ pollen grains per square meter, both varying significantly between months and zones.

Urticaceae pollen is present in the atmosphere year round, and concentration values of airborne pollen are highest in Spring. Correlation between airborne pollen values and pollen production is significant in the three study areas.

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Studies into the relationship between phenology and airborne pollen production of allergenic species are rather scarce. They are mainly focused on woody species (Latorre & Bianchi 1998), and little is known about herbaceous species from families as Poaceae and Urticaceae, which are among the most important causes of the respiratory allergy in the Mediterranean area (Subiza et al. 1992).

In peninsular Spain, the family Urticaceae accounted for 8 to 13% of the total annual airborne pollen counts obtained by means of Hirst traps at 15 widespread sites during the 1992–1998 period (Belmonte et al. 1999). In some of these places it is the most common pollen type in the airborne spectra (Moreno-Grau et al. 1998). In a previous study where prospected area also included Maó and Ciutadella in Minorca have shown the highest total counts of Urticaceae pollen among several Spanish localities, studied using the Cour method (Belmonte & Roure 1991, Belmonte et al. 1995).

Among the Urticaceae species present in Catalonia, *Parietaria judaica* L. is one of the most abundant (Bolós et al. 1990). It is widespread throughout the country and especially common in urbanised areas. It grows in wall fissures and disturbed soils, usually close to human habitats. Despite its allergenic significance, no previous research has been carried out on *P. judaica* phenology and pollen production.

Aerobiological records may not be detailed at genus level or at species level for the Urticaceae family, because pollen morphology of this family presents low variability (only

Urtica membranacea can be clearly distinguished). Records of airborne pollen in 15 Spanish localities over a 7-year period (1992–1998) revealed that Urticaceae pollen grains are present year round and that pollination periods may differ from place to place (Belmonte et al. 1999). These aerobiological differences may be attributed to variation in floristic composition of Urticaceae between areas. However, they may also be attributed to differences in length and distribution of the flowering period of the same species under different climatic conditions (Sparks & Carey 1995), or due to intra-specific variation in pollen production per plant (Vonhof & Harder 1995). Quantitative phenological studies are necessary to identify and characterize variations in floral phenophases and pollen production between populations of the same species and their relationship with concentrations of the airborne pollen.

Previous studies on the pollen production of anemophilous species have shown considerable variation among genera and among species (Subba Reddi & Subba Reddi 1986, Tormo Molina et al. 1996), but disparity of results have been obtained when intra-specific variation was analysed (Young & Stanton 1990, Thompson et al. 1989, Poulton et al. 2001). *Parietaria judaica*, with flowering phase lasting all year long, offers a valuable opportunity to study this intra-specific variability, as the same populations may be sampled repeatedly each month during a complete annual cycle.

In this study we analyse variability in phenology and pollen production of geographically distant populations of

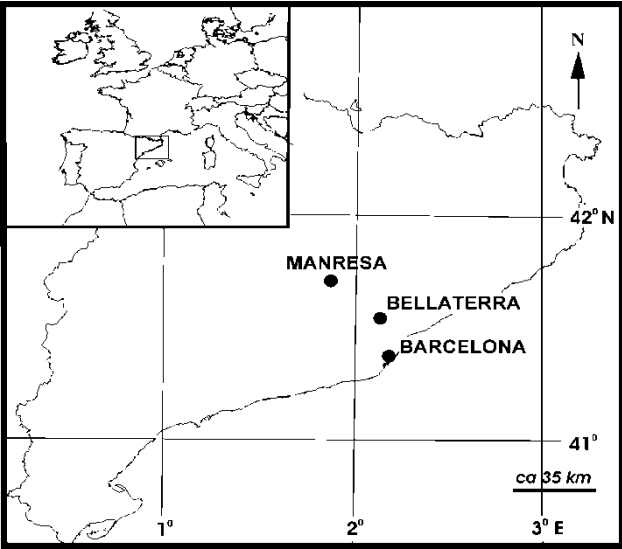


Fig. 1. Location of the three prospected zones: Barcelona, Bellaterra and Manresa in Catalonia (North East Spain).

Parietaria judaica and discuss the relationships between pollen production and the airborne pollen *dynamic* curves.

MATERIAL AND METHODS

Species studied

Parietaria judaica L. (= *P. officinalis* L. ssp. *judaica* (L.) Béguinot = *P. diffusa* Mert. et W.D.J. Koch; *P. judaica* hereafter) is a perennial herb, 10–50 cm tall. Each individual may consist of one to more than 50 shoots, most of them flowering copiously. Flowers are arranged in axillary inflorescences from the base to the top of the shoots. Each inflorescence habitually consists of a central female flower surrounded by three to six hermaphroditic flowers. The perianth comprises one whorl of sepaline tepals. It is cylindrical and 4-toothed in female flowers and 4-partite in hermaphroditic flowers. The androecium consists of four stamens inflexed in buds. Pollination is anemophilous, and pollen emission occurs when the filaments of the stamens reflex violently (Proctor & Yeo 1973). The gynoecium is superior and consists of one uni-carpellar pistil, which is shortly stylate with a large, brush-like stigma. Female flowers mature first, and the hermaphroditic flowers are protogynous. Anthesis starts first in the basal inflorescences and continues to the upper part of the shoot. Flowering may occur in very young plants.

Table I. Geographical and meteorological data of sample area.

Meteorological data have been obtained as yearly observed mean values of 22, 24 and 34 years in Manresa, Sabadell (close to Bellaterra) and Barcelona, respectively.

Locality	Geographic coordinates	Altitude (m a.s.l.)	Area (km ²)	Population density (p/km ²)	Mean annual temperature (°C)	Annual rainfall (mm)
Barcelona	41°24'N; 02°11'E	12	100,4	14 995	16.4	593
Sabadell	41°33'N; 02°07'E	82	37,9	4887	15.2	594
Manresa	41°43'N; 01°50'E	238	41,7	1535	13.6	619

Site description

Three sites have been selected to compare inter-population variation. Located in Barcelona, Bellaterra and Manresa (Fig. 1) environmental conditions vary between sites. Barcelona is a big city located on the littoral zone. Bellaterra is a semi-urbanised neighborhood near Sabadell, with natural woodland vegetation and agricultural areas nearby, and separated from the coast and from Barcelona by the Collserola Mountains (512 m a.s.l.). Manresa is a medium-sized city in the centre of Catalonia, surrounded mainly by agricultural areas.

Geographical and climatic characteristics of each site vary also (Table I). Long series of climatic data from Bellaterra are not available (only three years), and therefore data from Sabadell (the nearest city with climatic records, five km from Bellaterra) have been used (Allue Andrade 1990). Although all three sites have a Mediterranean climate, the mean annual temperature of Barcelona is 3°C higher than the mean annual temperature of Manresa, and Sabadell has an intermediate value. Mean annual rainfall increase follows the inverse pattern, with the Manresa value higher than that of Sabadell and Barcelona. Different monthly rainfall distributions have been observed between localities (Fig. 2), especially in Summer, when precipitations in Manresa are higher than those in the other two localities.

Phenology and pollen production

At each of the three sites, three populations of *P. judaica* have been selected (a total of nine plots), all of which are located at the base of more or less fissured old walls in urbanised areas. These populations are usually mono-specific and conform to a rather narrow band (up 1 m wide) following the fissures of the wall. The sampling area was

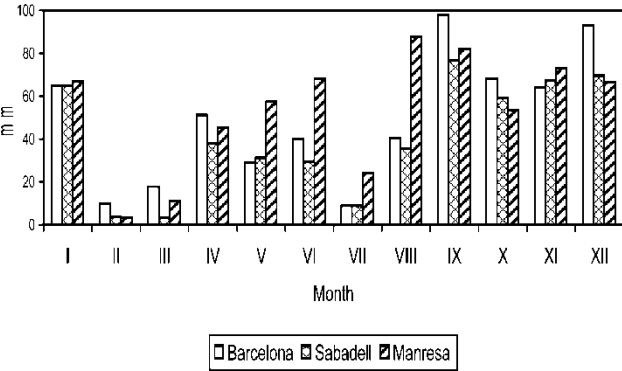


Fig. 2. Mean monthly precipitation corresponding to 1995–1999 period for the three prospected areas: Barcelona, Sabadell and Manresa. (For Bellaterra we used data from Sabadell, the nearest city from Bellaterra with precipitation records).

delimited by a transect 10.0×0.5 m long, adjacent to the corresponding wall. Each of the populations was sampled seven times from November 1999 to October 2000, with a variable frequency according to the seasonal dynamics of the plants (Table II). From December 1999 to February 2000, plots were visited to record phenological status of plants, but as population density did not show any apparent increase, no counts were performed until March 2000. For the same reason, no data were recorded for April and August 2000. Unfortunately, as *P. judaica* is considered a weed, several monitored populations were harvested during our study, and new populations had to be chosen to replace them (Table II).

Phenology was based on periodical recording of number of reproductive and vegetative shoots in the nine plots. Counts were performed on twenty contiguous 50×50 cm quadrats arranged along the 10.0×0.5 m transect for each population. To perform laboratory measurements, ten reproductive shoots were recollected regularly every 25 cm in an area next to the sampled one.

The number of hermaphroditic flowers on each of these ten reproductive shoots was counted in the laboratory under a stereoscopic microscope. Pollen production estimates were performed following the methodology proposed by Cruden (1977) and were adapted to the unique characters in *P. judaica*. One randomly selected, closed flower of each shoot was removed to perform pollen production estimates. One undehiscent anther of each flower was harvested and individually stored in a microcentrifuge tube with 0.3 ml of 70% ethyl alcohol until counting could be completed. To perform the counting the anther was dabbed with a needle and the tube containing it was shaken mechanically with a Vortex mixer for at least five minutes in order to homogenize the solution. The total number of pollen grains per anther was counted under a light microscope (100×) in a 1 mm³ (1×1×1 mm) counting chamber delimited on a slide. Three sub-samples per anther were counted, and the average of these calculated.

To obtain the total number of pollen grains per anther (N) we multiplied the average number of pollen grains counted in the 3 sub-samples $([n_1 + n_2 + n_3]/3)$ by the dilution factor ($d=300$). Multiplying the obtained value by the number of stamens per flower (always four), by the average number of hermaphroditic flowers per shoot (F_h) and by the average number of reproductive shoots by square meter (R) observed in the field, we obtained the mean pollen production per area (P).

$$P = ([n_1 + n_2 + n_3]/3) \times d \times 4 \times F_h \times R$$

An analysis of the variance (ANOVA) was performed with the SPSS (9.0) statistical package, to test differences between the three study sites during a complete annual cycle. The univariate linear

model included total density, the percentage of reproductive shoots, the number of hermaphrodite flowers, the number of pollen grains per anther and the pollen production as dependent variables, and site and month as fixed factors.

Airborne pollen

Airborne pollen values were obtained using seven-day volumetric spore Hirst traps (Hirst 1952), following the method adopted by the Spanish Aerobiology Network (Dominguez et al. 1991).

The basic data are mean daily pollen concentrations expressed as the number of pollen grains per cubic meter of air (p/m³). For each station and month, the sum of the mean daily concentrations of all days in the corresponding month (monthly index) has been calculated. Non-parametric Spearman's rank correlation was calculated to assess associations between monthly mean pollen production of *P. judaica* and Urticaceae monthly airborne pollen, also with the SPSS (9.0) statistical package.

RESULTS

Phenology and pollen production

Parietaria judaica shows more or less active vegetative growth year round, but intensity of this phenological phase decreases in Winter and in Summer. Many small shoots start with fast vegetative growth at the end of the Winter, some of them attaining more than 50 cm in height during Spring. Simultaneously, shoots enter the reproductive phase, producing axillary inflorescences from bottom to top. Anthesis is the phenological dominant phase during Spring, and at the end of this season most shoots lose almost all their leaves, producing a considerable accumulation of dead biomass. Fruit maturation and dispersal occur mainly in Summer. In Autumn, a second active vegetative growth phase is not unusual, and finally, most of the plants enter into a resting phase lasting until next Spring.

Vegetative and reproductive shoots are present all the year round in the three study sites. Total density (Fig. 3) ranged from 168 to 522 shoots per m², with peaks in May, in Bellaterra and Barcelona, and in July in Manresa, where mean total density reached the maximum absolute value. Total density is significantly different between sites ($F_{2, p<.000}=44.031$) and between monthly records ($F_{6, p<.000}=6.297$), and interaction between both variables ($F_{12, p<.000}=7.115$) is also significant, that is, differences

Table II. Code of plots sampled at each location.

The first number appearing following the three letters code serves as an indicator of the plot; several monitored populations were harvested during our study and new populations had to be chosen to replace them: the second number of the code indicates if it is the first selected plot (number 1), the second selected plot to replace the previously destructed one, etc (until fifth).

Months	Barcelona			Bellaterra			Manresa		
	BCN1	BCN2	BCN3	BEL1	BEL2	BEL3	MAN1	MAN2	MAN3
XI-99	BCN1-1	BCN2-1	BCN3-1	BEL1-1	BEL2-1	BEL3-1	MAN1-1	MAN2-1	MAN3-1
III-00	BCN1-1	BCN2-1	BCN3-1	BEL1-1	BEL2-1	BEL3-2	MAN1-1	MAN2-2	MAN3-1
V-00	BCN1-1	BCN2-2	BCN3-1	BEL1-1	BEL2-1	BEL3-3	MAN1-1	MAN2-3	MAN3-2
VI-00	BCN1-2	BCN2-2	BCN3-1	BEL1-1	BEL2-2	BEL3-4	MAN1-1	MAN2-3	MAN3-3
VII-00	BCN1-2	BCN2-2	BCN3-1	BEL1-1	BEL2-3	BEL3-4	MAN1-1	MAN2-3	MAN3-3
IX-00	BCN1-2	BCN2-2	BCN3-1	BEL1-1	BEL2-4	BEL3-5	MAN1-2	MAN2-3	MAN3-3
X-00	BCN1-2	BCN2-3	BCN3-1	BEL1-1	BEL2-5	BEL3-5	MAN1-2	MAN2-3	MAN3-3

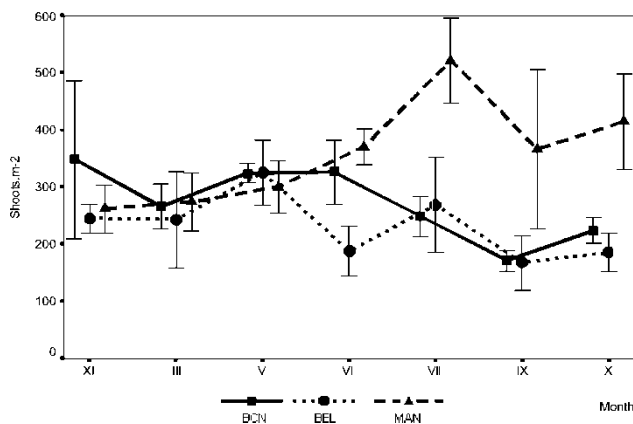


Fig. 3. Mean total density (shoots.m⁻²) per zone from November 1999 to October 2000 (in December, January, February, April and August samples were not performed). BCN - Barcelona, BEL - Bellaterra, MAN - Manresa. Abbreviations applied here subsequently used in all text figures.

between months of the same site or differences between sites in the same month.

The number of reproductive shoots increase gradually from November to May in Barcelona and Bellaterra and from November to July in Manresa (Fig. 4). The percentage of reproductive shoots changes significantly within sites and during the year (Table III), as illustrated by the delayed pattern observed in Manresa. At this site, the number of reproductive shoots continues to increase until July, when the number of reproductive shoots is more than twice the numbers recorded for Bellaterra and Barcelona.

The number of hermaphroditic flowers per shoot follows the same pattern in the three areas (Fig. 5), except in Manresa in October. Minimum values occurred in November (mean of 15 to 30 flowers per shoot) and a maximum was observed in March, with mean values ranging from 71 to 79 flowers per shoot. Differences are also significant between the sites, but were not significant between months and when interaction between these two factors was considered (Table IV).

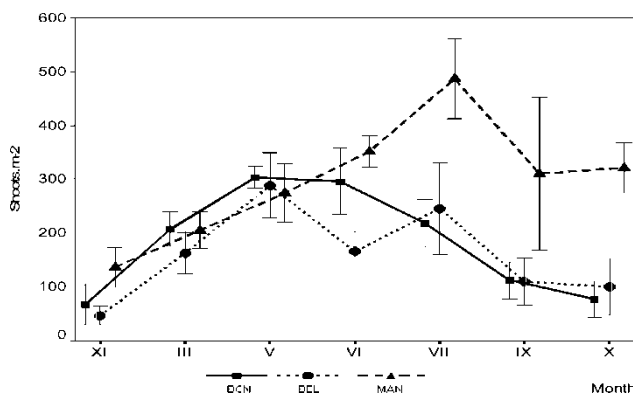


Fig. 4. Average number of reproductive shoots (shoots.m⁻²) per zone and per month from November 1999 to October 2000 (in December, January, February, April and August samples were not performed).

Table III. Results of the univariate analysis of the variance performed with percentage of reproductive shoots as dependent variable and zone and month as fixed factors.

df – degree freedom; F – Fisher value; P – probability

Source of variation	df	F	P
Zone	2	6.052	.005
Month	6	21.339	.000
Zone*Month	12	1.175	.332

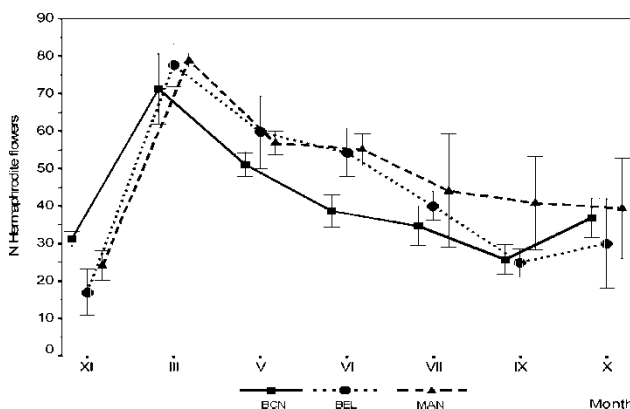


Fig. 5. Mean number of hermaphroditic flowers per shoot in each prospected zone from November 1999 to October 2000 (in December, January, February, April and August samples were not performed).

The number of pollen grains per anther ranges from 35 744 to 86 333 and follows a similar annual dynamic pattern for the three sites (Fig. 6). The maxima were reached in March in Barcelona and in May in Manresa and Bellaterra. This last site presents the lowest value. ANOVA analysis performed with sites and months as fixed factors revealed that both these and their interaction are significant (Table V).

The average pollen production is $2\,350 \times 10^6$ pollen grains per m², with high variability between sites and plots (Fig. 7). The highest mean pollen production was attained between March and May in Manresa and Barcelona and in May in Bellaterra, where increases between consecutive months were less noticeable. Pollen production from March to October 2000 is always higher in Manresa than in Barcelona and Bellaterra. Significant pollen production variability has been detected between sites and months with ANOVA analysis of

Table IV. Results of the univariate analysis of the variance performed with average number of hermaphrodite flowers per shoot as dependent variable and zone and month as fixed factors.

df – degree freedom; F – Fisher value; P – probability

Source of variation	df	F	P
Zone	2	1.675	.000
Month	6	16.617	.200
Zone*Month	12	0.611	.820

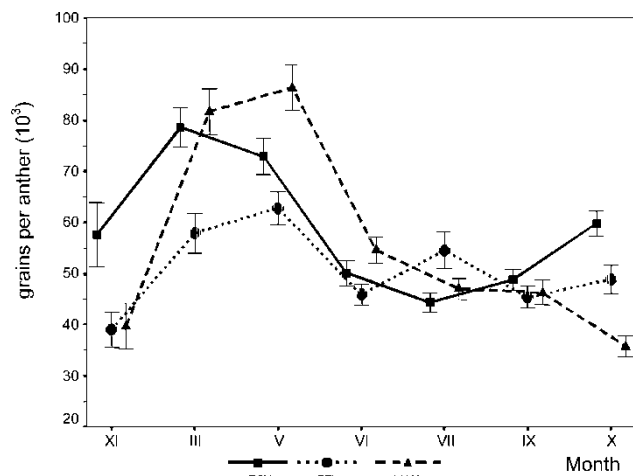


Fig. 6. Mean number of pollen grains per anther in each sampling zone from November 1999 to October 2000 (in December, January, February, April and August samples were not performed).

Table V. Results of the univariate analysis of the variance performed with mean number of pollen grains per anther as dependent variable and zone and month as fixed factors.

df – degree freedom; F – Fisher value; P – probability

Source of variation	df	F	P
Zone	2	3.634	.035
Month	6	12.688	.000
Zone*Month	12	2.155	.033

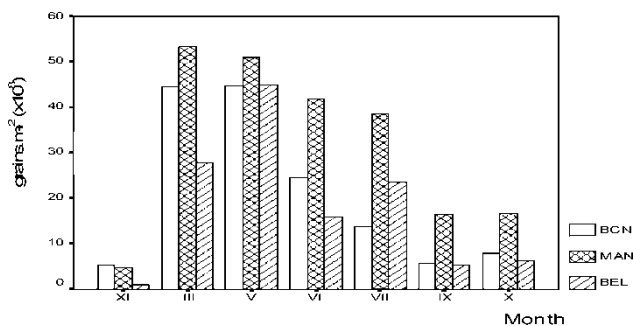


Fig. 7. Average pollen production (number of pollen grains per square meter) in each prospected area from November 1999 to October 2000 (in December, January, February, April and August samples were not performed).

the average pollen production, but the interaction between these two factors did not prove to be significant (Table VI).

Airborne pollen

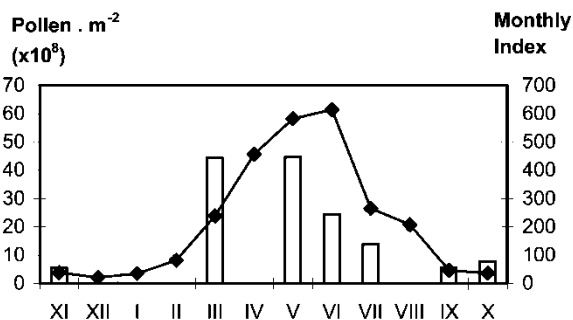
The annual dynamics of the airborne pollen values fitted relatively well with that detected in pollen production of plants (Fig. 8). Urticaceae pollen is present in the atmosphere throughout the year and concentration curves of the airborne pollen produced a well-defined peak.

Table VI. Results of the univariate analysis of the variance performed with average pollen production as dependent variable and zone and month as fixed factors.

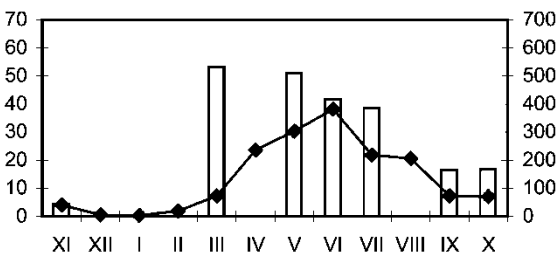
df – degree freedom; F – Fisher value; P – probability

Source of variation	df	F	P
Zone	2	9.615	.000
Month	6	21.514	.000
Zone*Month	12	0.894	.560

A



B



C

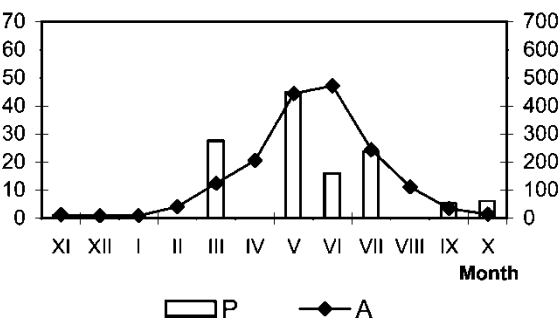


Fig. 8. Mean pollen production (P - number of pollen grains per square meter) of *P. judaica* and monthly total airborne pollen (A - monthly index) in A - Barcelona, B - Manresa, C - Bellaterra, from November 1999 to October 2000 (mean pollen production was not recorded in December 1999 and in January, February, April and August 2000).

Nevertheless, this maximum occurs at least one month later than the maximum pollen production recorded for the *P. judaica* plants. Correlation between airborne pollen values and pollen production is significant in the three sites (Table VII).

Table VII. *Spearman's coefficient of correlation (r) and its significance (P) for the three prospected areas.*

Sites	r	P
Barcelona	0.7143	0.036
Bellaterra	0.6307	0.064
Manresa	0.7143	0.036

DISCUSSION

Parietaria judaica has a similar phenological pattern in the three sites studied: total density of shoots increases progressively from March to the beginning of Summer, and flowering shoot density curves follows similar dynamics with a maximum in May or July. These annual dynamics coincide with that observed in many perennial herbs of the Mediterranean region, where Spring is the most favorable season for plant growth and reproduction (Pacini & Franchi 1984, Guardia et al. 1998). Nevertheless, some differences occur. Firstly, the flowering phase is continuous year round, although with very few flowering shoots during the Winter months. Secondly, almost all shoots enter the flowering phase and usually produced many flowers. This long blooming period together with the high flower production are life-history attributes that have been associated with plants of disturbed habitats (Grime 1979), and therefore are expected characteristics for a ruderal species such as *P. judaica*.

The mean total density of shoots follows a very similar curve for the Barcelona and Bellaterra sites, but a significantly delayed peak is observed in the Manresa plots. Low Winter temperatures prevent active plant growth. Manresa, located inland and at a higher latitude and altitude, has cooler Winters than Barcelona and Bellaterra. Consequently plant growth started later. Moreover, Summer drought is less noticeable in Manresa, resulting in plants extending their vegetative growth period during this season. Intra-specific variation of the phenological behaviour along climate gradients has been detected for many plants (Orshan 1989, Castro-Díez & Montserrat 1998, Beatley 1974). Larger datasets of phenological records are needed to elucidate the nature of relationships between phenology and climatic factors (Sparks & Carey 1995).

The mean number of flowers per shoot follows the same annual dynamics in the three study sites, with a maximum in March. No differences were detected for this variable in the Manresa site in July, such as those found with total and reproductive density values. This reflects that shoots of *P. judaica* have a homogeneous structure: populations of *P. judaica* differ in the number of shoots they produce, but not in the basic structure of these shoots. Wan et al. (2002) found similar results for *Ambrosia psilostachya*. With experimental warming they detected a significant increase of the total pollen production per unit of ground area, which resulted primarily from the increase in stem numbers and not from increased pollen production per stem.

The average number of pollen grains per anther of

P. judaica is $54\,950 \pm 2\,103$. This value appears relatively high when compared to those of anemophilous trees (Tormo Molina et al. 1996) or those recorded for other herbaceous species (Subba Reddi & Subba Reddi 1986, Vonhof & Harder 1995, Etterson & Galloway 2002). Nevertheless, heterogeneity of the applied methodologies between these studies makes it difficult to extract general conclusions.

Although the number of pollen grains per anther presents considerable variability between individuals, all populations followed the same annual dynamics during the study period. A unimodal curve with maximum pollen production occurs in Spring when climatic conditions are the most favorable in the Mediterranean region. Our results indicate that the amount of pollen per anther is not genetically fixed as has been suggested by several authors (Subba Reddi & Subba Reddi 1986) as it varies significantly between sites and throughout the year. The fact that all populations studied show the same annual dynamics emphasized the consistency of this pattern. A number of recent studies have shown that environmental conditions, such as herbivory and soil fertility, can also affect pollen production in plants by altering pollen production per flower (Delph et al. 1997, Lau et al. 1995). The number of pollen grains produced by an individual is probably subjected to a trade-off with pollen size and restrictions due to environmental conditions. Pollen grains, like seeds, require resources from the parent plant in order to mature, germinate and initiate growth. As resources available for male function are limited, a positive relationship between environmental factors and pollen production is predictable.

Regarding pollen production per surface unit, values obtained for *P. judaica* were extremely high compared to those obtained for other species (Hidalgo et al. 1999). These values have to be interpreted taking into account the spatial distribution of *P. judaica*, with scattered, locally dense populations. Therefore, pollen production values should take into account the frequency of populations over each study area.

Production of airborne pollen of Urticaceae increases gradually from March to June at all three sites. This pattern fits with data observed for the same family in Genoa (Arobba et al. 1992), a city also located in the Mediterranean basin. These authors found a high and significant correlation between the sum of the mean daily temperatures and the number of the days from the first of January to the date of the beginning of the main pollen season.

There is a strong similarity between annual airborne pollen dynamics and the annual pattern observed for pollen production of plants, but with a delay of at least one month for the airborne pollen curve. Maximum airborne pollen values occur in June while maximum pollen production is in April or May. Another divergence between concentration of airborne pollen and pollen production is the ranking of the relative position between sites. Barcelona shows the highest airborne pollen concentrations almost all the year round, except for September and October, when Manresa shows maximum value. Instead, the highest plant pollen production was observed in Manresa from March to September, especially in June and July.

These divergences may have two different explanations, which are not mutually exclusive. First, morphological traits of *P. judaica* suggest that most of the pollen produced by this plant remains at a relatively short distance from the source. The possibility that pollen produced by *P. judaica* reaches local pollen traps (located at 20–30 m from ground level) mainly depends on convection air streams. Consequently, pollen captured in the atmosphere is probably the sum of pollen released from the plants throughout a period of several days (re-flotation).

The divergence of pollen peaks might also be a result of different level of precision employed to obtain the data. Pollen production has been estimated at species level, but airborne pollen measurements are performed at the family level. The family Urticaceae comprises three genera and ten species in the area (Belmonte et al. 1999), some of these, such as species of *Urtica*, being relatively abundant and with blooming phase that are not totally coincident with that of *P. judaica*. Therefore, airborne pollen values comprise not only *P. judaica* pollen but also other Urticaceae pollen.

CONCLUSIONS

Parietaria judaica presents a characteristic Mediterranean phenological pattern in the three sites studied, with main vegetative and reproductive activity in Spring, when climatic conditions are the most favorable for plant physiological activity.

The number of pollen grains per anther varies significantly throughout the year. This may be due to a positive correlation with environmental conditions. Differences detected between the three prospected zones would ratify this correlation.

The pollen production values obtained for *P. judaica* are relatively high when compared with other species. This could be due to the ruderal habitat of *P. judaica*.

There is a significant correlation between the annual variations in the concentrations of airborne pollen of Urticaceae and the annual pattern observed for pollen production of *P. judaica*. Nevertheless, several divergences were detected, which may be attributed to the contribution of other Urticaceae species to the airborne pollen concentration. Also re-flotation may result in deviations.

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