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ORIGINAL ARTICLE

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Male phenology of three species of *Cupressus*: correlation with airborne pollen

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Abstract A 3-year male phenological field study was performed on the represented species of the genus *Cupressus* in the city of Cordoba (Spain): *Cupressus arizonica*, *C. macrocarpa* and *C. sempervirens*. A new and complete description of the phenological stages of the male flower of *Cupressus* was obtained. Five phenological phases were described using internal and external bud/flower traits. In general, different pollination periods were recorded for the 3 years. *C. arizonica* flowered from 20 to 23 days before the others. *C. macrocarpa* and *C. sempervirens* flowered almost simultaneously, although the former appeared to flower slightly earlier. The total number of trees per square kilometre was estimated taking into account the total number of trees of each species in the city and surrounding area (5 km radius from the city centre). Data corresponding to total pollen production per tree were taken from a previous study in which the partial contribution of each species to atmospheric pollen was estimated. Considering all these parameters, a theoretical airborne pollen model was proposed. A correlation coefficient (R^2) of 0.46 was obtained when comparing this model with the average airborne pollen concentrations for the last 18 years. According to the proposed model, *C. macrocarpa* trees accounted for 78% of total airborne pollen, while *C. sempervirens* and *C. arizonica* accounted for only 18% and 4%, respectively. The final objective of this study was to provide additional biological information on these species responsible for winter pollinosis in the Mediterranean area. Forecasting pollen emission and dispersion has an important application in public health warnings.

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Introduction

Phenology is generally described as "the art of observing life cycle phases or activities of plants and animals in their temporal occurrence throughout the year" (Lieth 1974). Phenology has recently emerged as an important focus of ecological research (Schwartz 1999). Phenological data are a potential contribution to research into climatic changes (Schwartz 1999), flowering being the stage most sensitive to climatic changes (Spano et al. 1999). Phenology is usually studied by subdividing the development of biological taxa into identifiable sections along a time axis. These subdivisions are called phenophases and are used to monitor the development of the life cycle of an organism. Relevant events for trees are the moment of budburst, leaf elongation and abscission or flowering time.

The application of floral phenology data is of interest in pollen allergy studies. By charting the phenology of allergenic plants, we can detect the start of airborne allergen emission. In such studies, two types of phenological data can be considered: direct observation of plants and measurement of airborne pollen emitted by these plants, indicating the timing of flowering of populations. The airborne pollen spectrum reflects the composition of local vegetation. This spectrum depends on a multitude of factors, the most important being meteorological conditions, pollen grain dispersal capacity, plant numbers and pollen production per individual. The location of the aerobiological samplers is sometimes the most important factor to be taken into account when interpreting the pollen spectrum. The proximity of the pollen source or the wind trend may distort the pollen spectrum. Consequently, the assumption that airborne pollen is equivalent to phenology is misleading, particularly in regions with variable climatic conditions (Keynan et al. 1989). However, it could afford a practical

method for quantifying male phenology. This means that aerobiological studies should be improved through field phenological observations in order to provide more accurate information on the airborne pollen spectrum.

Gymnosperms have male reproductive structures in cones/strobili with microsporophylls arranged spirally through the central axis. Cupressaceae is the only family that shows a non-spiral (cyclic) arrangement of microsporophylls. Each microsporophyll bears a variable number (2–6) of sporangia (pollen sacs). Pollen is produced in large quantities (Hidalgo et al. 1999) and dispersed by wind in *Cupressus*, the surrounding areas being covered by a yellow dust.

Only three species of *Cupressus* are present in the city of Cordoba: *C. sempervirens* L. (common cypress, originally from the eastern Mediterranean area), *C. macrocarpa* Hartweg (Monterrey cypress, originally from North America) and *C. arizonica* Greene (Arizona cypress, originally form the south of the United States and the north of Mexico). *C. sempervirens* produces two different kinds of branches, depending on the variety. All common cypress trees from the city of Cordoba belong to *C. sempervirens* var. *pyramidalis* L.

Cupressaceae pollen has been shown to be responsible for winter pollinosis in the Mediterranean area when no other plants are flowering (Caramiello et al. 1991; D'Amato and Liccardi 1994). Furthermore, this type of pollen is very common in the air over Cordoba, accounting for at least 30% of the total pollen count during the winter season (Ruiz de Clavijo et al. 1988). A positive reaction was observed in 35% of pollinosis patients in the city of Cordoba (Guerra et al. 1996). Species of the *Cupressus* genus have often been reported to be responsible for pollinosis (Ford et al. 1991; Mari et al. 1997; Barletta et al. 1998).

The Cupressaceae family produces pollen grains characterized as spheroidal, intectate and monoporate, with a distinct annulus (Bortenschlager 1990). Cupressaceae pollen grains have never been used as a diagnostic trait for distinguishing between genera or species as they share the same characteristics (stenopalynous character) under light microscopy and even under scanning (Bortenschlager 1990) or transmission electron microscopy (Kurmann 1994). All Cupressaceae/Taxaceae species are included in the same pollen type in aerobiological studies (Galán et al. 1998a). Consequently, the partial contribution of each Cupressaceae species to the airborne pollen spectrum cannot be determined. Although species of three genera of Cupressaceae (*Cupressus*, *Thuja* and *Juniperus*) are relatively common in the province of Cordoba, only those of *Cupressus* are sufficiently common to produce enough airborne pollen during air sampling.

The objectives of this paper were: (1) to describe phenological phases in the genus *Cupressus* using internal and external traits, (2) to study a 3-year period of phenological data in the different species of *Cupressus* 5 km around the city of Cordoba, and (3) to correlate the temporal distribution of the blossoming period in these species with Cupressaceae airborne pollen. The overall aim was to provide additional biological information on these species in order to forecast pollen emission and dispersion once the potential pollen production of each species (Hidalgo et al. 1999) and the bioclimatic factors affecting flowering (Galán et al. 1998b) had been determined. The establishment of a forecasting system has important applications in public health warnings.

Materials and methods

The city of Cordoba is located in the southwest of the Iberian Peninsula (37°50'N, 4°45'W), 123 m above sea level. Cordoba has a typical Mediterranean climate, with a weak continental influence. Average annual rainfall is 670 mm and the average yearly temperature is 18°C. For this study, the authors selected 227 mature and well-developed individuals of the represented species of the genus *Cupressus* distributed in the city and surroundings: *C. arizonica*, 53 individuals; *C. sempervirens*, 54 individuals; *C. macrocarpa*, 120 individuals. Other Cupressaceae species, such as *Thuja or Juniperus*, not included in this study, are very scarce in the city.

Phenological survey

The male flower phenology study was carried out on the selected *Cupressus* individuals during the winter–spring of 1998–2001. Fieldwork consisted of weekly visits to the 227 trees during the winters and springs of 1998–2001. Twice-weekly visits were made during the main flowering period. Previous experience on this genus and other studied genera indicated that this sampling procedure is the best way to assess phenological evolution. On each visit, the stage and size of the male buds/flowers of all the trees were observed. A sample of this flower was photographed and examined as described in the section on cytological observations, in order to help define the phenophases. Phenological phases were stabilized according to the steps proposed by Lieth (1974), with some modifications. Thirteen flowers per tree were measured during each visit. Once the phenological phases of the bud/flower had been defined, the phenological stage of the trees was evaluated, taking into consideration the most frequent phenophase of the pool of flowers around the crown.

Cytological observations

Buds and flowers from *C. sempervirens*, *C. arizonica* and *C. macrocarpa* were taken from several trees at different stages of development. The flowers were measured and fixed with 1:1:8 FAA (formol: acetic acid: alcohol). Early stages of microsporogenesis (pre-meiotic stages) were observed by embedding the flowers in methacrylate (JB4). Semi-thin sections were cut with a glass knife in a Reichert Jung Autocut, stained with toluidine blue and observed by light microscopy. Post-meiotic stages were directly observed by light microscopy by crushing the flowers in a dilute solution of toluidine blue and basic fuchsin.

Estimation of relative pollen production per species

In order to estimate the relative contribution of each species to total Cupressaceae airborne pollen, the total number of trees per square kilometre was determined by counting the total number of trees of each species in the city and in the surrounding area (5 km radius). These values were multiplied by the total pollen production per tree. The results were converted into percentages in order to determine the relative contribution of each species. The total pollen production per tree was described by Hidalgo et al. (1999).

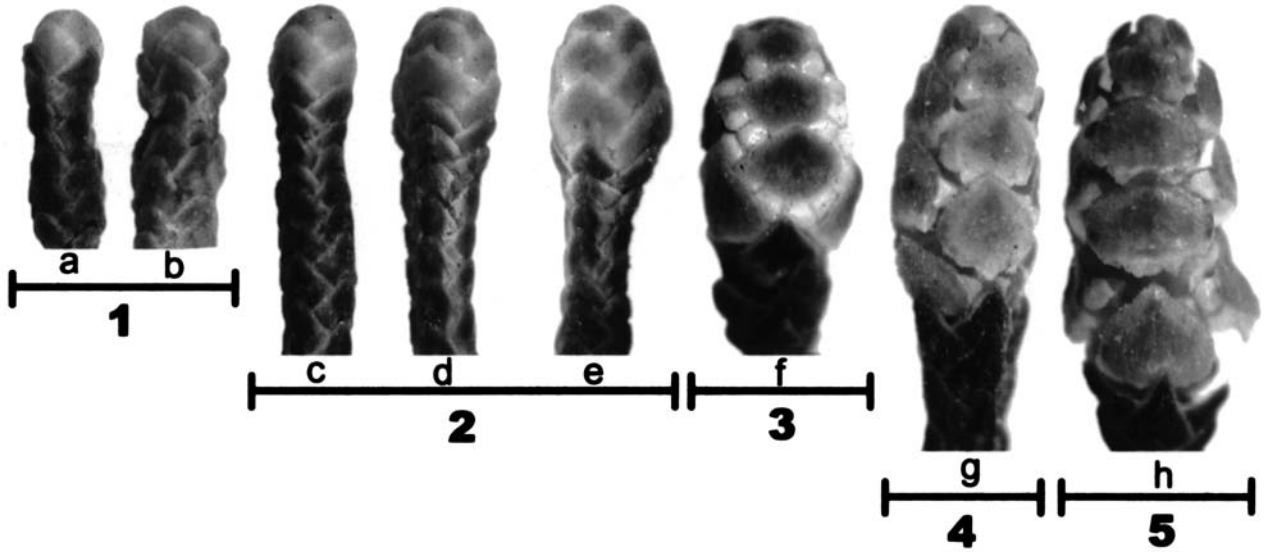

Fig. 1a–h Male phenological phases in *Cupressus arizonica*. Phase 1, bud differentiation (**a**, **b**); phase 2, immature flowers (**c–e**); phase 3, near to flowering (**f**), notice the pollen sac appearing between the scales; phase 4 blossoming flower (**g**), the pollen sacs are opened and the flower are emitting pollen. The scales can be opened or closed depending on relative humidity. Phase 5, senescent flower (**h**), the pollen sacs are empty and the scales are completely unfolded. ×8.5

Table 1 Phenological phases (phenophases) in the three studied species of *Cupressus* and male flower length in each phenophase. Mean, standard deviation and range are shown

Phenophase	Description	Male flower size (mm)		
		C. sempervirens	*C. macrocarpa*	*C. arizonica*
1	Male bud differentiation	1.2±0.3 (0.9–1.7)	1.2±0.4 (0.7–1.7)	1.1±0.3 (0.9–1.7)
2	Immature flowers	3.0±0.5 (2.0–4.0)	3.6±0.9 (2.1–5.0)	2.5±0.5 (1.8–3.3)
3	Near to flowering (change of colour)	4.9±0.5 (4.2–5.8)	6.1±0.3 (5.5–6.5)	4.3±0.3 (4.0–4.8)
4	Full flowering (the flowers are open in the main)	7.0±1.0 (5.5–9.0)	8.2±0.9 (7.2 9.5)	5.7±0.5 (5.0–6.0)
5	Senescent period (the flowers are senescent in the main)	7.9±1.0 (7.5–9.0)	8.6±0.5 (8.0–9.0)	6.7±0.4 (6.0–7.3)

Aerobiological and meteorological data

Aerobiological data were obtained from the Aerobiology Spanish Network (REA) databank. These data were collected using a 7-day spore trap (Hirst 1952) placed on the roof of the Faculty of Sciences, University of Cordoba, approximately 15 m above ground level. The standard sampling procedures proposed by REA (Domínguez et al. 1991) were used to obtain pollen counts. Data for 3 years of pollen monitoring records were studied and compared with field observations. Data from 19 years were used for the graphical representation of the annual average pollen curve. This curve was obtained after aligning the main peak in accordance with the method proposed by Rogers (1997). Meteorological data were supplied by the National Institute of Meteorology, based on readings taken at Cordoba Airport, which is located 5 km south of the pollen-sampling site.

Results

Phenological phases

Table 1 shows the male phenological phases based on the development of the pool of buds/flowers of the tree. Five different phenophases can be defined for the three *Cupressus* species. These phases are differentiated by size, colour, stage of microsporogenesis and blossoming behaviour of the androecium. Figure 1 shows the shape of the male bud/flower of the genus *Cupressus* based on the model of *C. arizonica*. Figure 2 represents the different stages of development of the pollen grains of the genus *Cupressus*, based on the *C. macrocarpa* model.

- Phenophase 1. Bud differentiation. In this phase the buds can be clearly seen at the top of the branch (Fig. 1a, b). Considering the whole tree, the average size of the buds was 1.2 mm in *C. sempervirens*, 1.2 mm in *C. macrocarpa*and 1.1 mm in *C. arizonica*(Table 1). During this phenophase, the different tissues involved in the microsporogenesis were differentiated (Fig. 2a).
- Phenophase 2. Immature flowers. The flowers are clearly developed but still immature (Fig. 1c–e). This phase is characterized by a lengthening of the immature flowers to 3.0 mm in *C. sempervirens*, 3.6 mm in *C. macrocarpa*and 2.5 mm in *C. arizonica*(Table 1). Phenophase 2 can be associated with the meiosis of the pollen mother cell (PMC) (Fig. 2b, c) and the early maturation of the microspore (Fig. 2d, e). Figure 2b

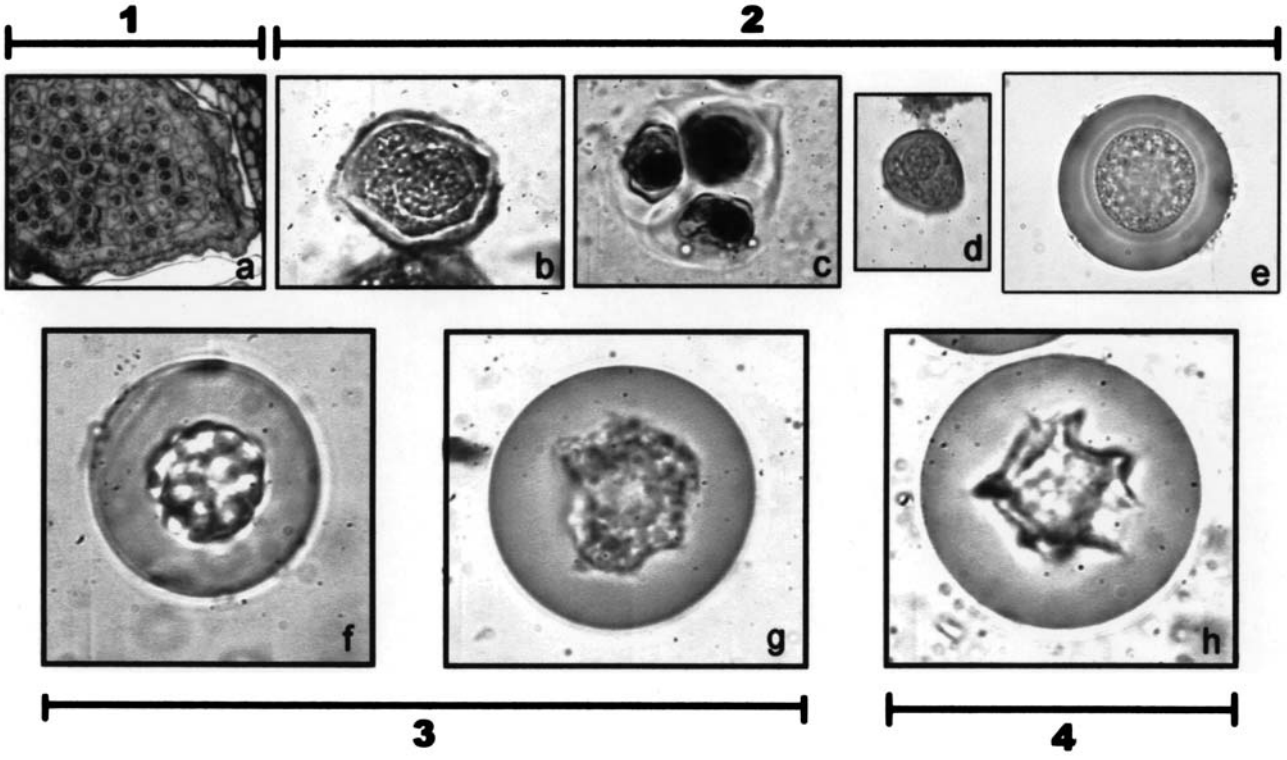

Fig. 2a–h Different stages of microsporogenesis in *C. macrocarpa* and correlation with phenological phases. **a** Undifferentiated tissue. **b** Pollen mother cell stage; synthesis of callosic envelope. This cell appears free due to the crushing of the pollen sac, adopting a more or less regular arrangement in the sporogenous tissue. **c** Tetrad stage (within callosic envelope). **d** Just-free microspore. **e** Early maturation of the microspore with exine and intine already formed. **f** Late maturation of the microspore during the nutrient accumulation phase. **g** Star-like cytoplasm transformation. **h** Mature pollen grain at anthesis. Phenological phase 1 corresponds to the start of tissue differentiation. Phenological phase 2 corresponds to meiosis and early maturation of the microspore (**b–e**). Phenological phase 3 coincides with late maturation (**f**, **g**). Phase 4 corresponds to mature pollen grains (**h**)

represents a PMC during the formation of the callosic envelope. Figure 2c corresponds to the tetrad stage. A just-free microspore after meiosis appears in Fig. 2d. This phase concludes with the early maturation of the microspore (Fig. 2e) in which the exine and the characteristic thick intine of Cupressaceae pollen is formed.

– Phenophase 3. Near to flowering. This is a short period in which the flowers change colour as the pollen sac appears through the scales (Fig. 1f). The colour in *C. arizonica*turns to an intense yellow and in *C. macrocarpa*and *C. sempervirens*to more or less white. In this phase, the immature flower swells due to the increase in size of the microspores. The length of the flower increases to 4.9 mm in *C. sempervirens*, to 6.1 mm in *C. macrocarpa*and to 4.3 mm in *C. arizonica*(Table 1). This phase consists in the late maturation of the microspores (Fig. 2f–h). Pollen maturation starts with the accumulation of nutrients (Fig. 2f). Finally, the pollen grains adopt a clear star-like shape in the cytoplasm (Fig. 2g) before transforming into mature pollen (Fig. 2h).
– Phenophase 4. Full flowering. The flowers open in the main and emitting mature pollen (Figs. 1g, 2h). The microsporophylls do not dehisce simultaneously. Dehiscence of the microsporophylls starts from the bottom of the flower and gradually opens towards the top of the flower. Figure 1g shows a *C. arizonica*flower at anthesis. In this flower, some microsporophylls at the bottom are senescent with empty sporangia, whereas others at the top are still closed. The scales in the microsporophylls at anthesis are opened when relative humidity (RH) is low and closed if humidity is high. The end of this phase consists in the senescence of the majority of the flowers. The rachis grows during the flowering phase to allow gradual emission of pollen by microsporophylls. Mean flower size was 7.0 mm in *C. sempervirens*, 8.2 mm in *C. macrocarpa*and 5.7 in *C. arizonica*(Table 1).
– Phenophase 5. Senescent period. All the flowers start the senescent period. This is the end of the pollen emission period. The pollen sacs remain in the flower but are now completely empty and the scales totally unfolded (Fig. 1h). After this phase, the flower turns dark brown, a colour typical of necrotic tissue. Finally, the flowers drop from the tree although they often remain on the tree for some weeks. Mean flower size was 7.9 mm in *C. sempervirens*, 8.6 mm in *C. macrocarpa*and 6.7 mm in *C. arizonica*(Table 1).

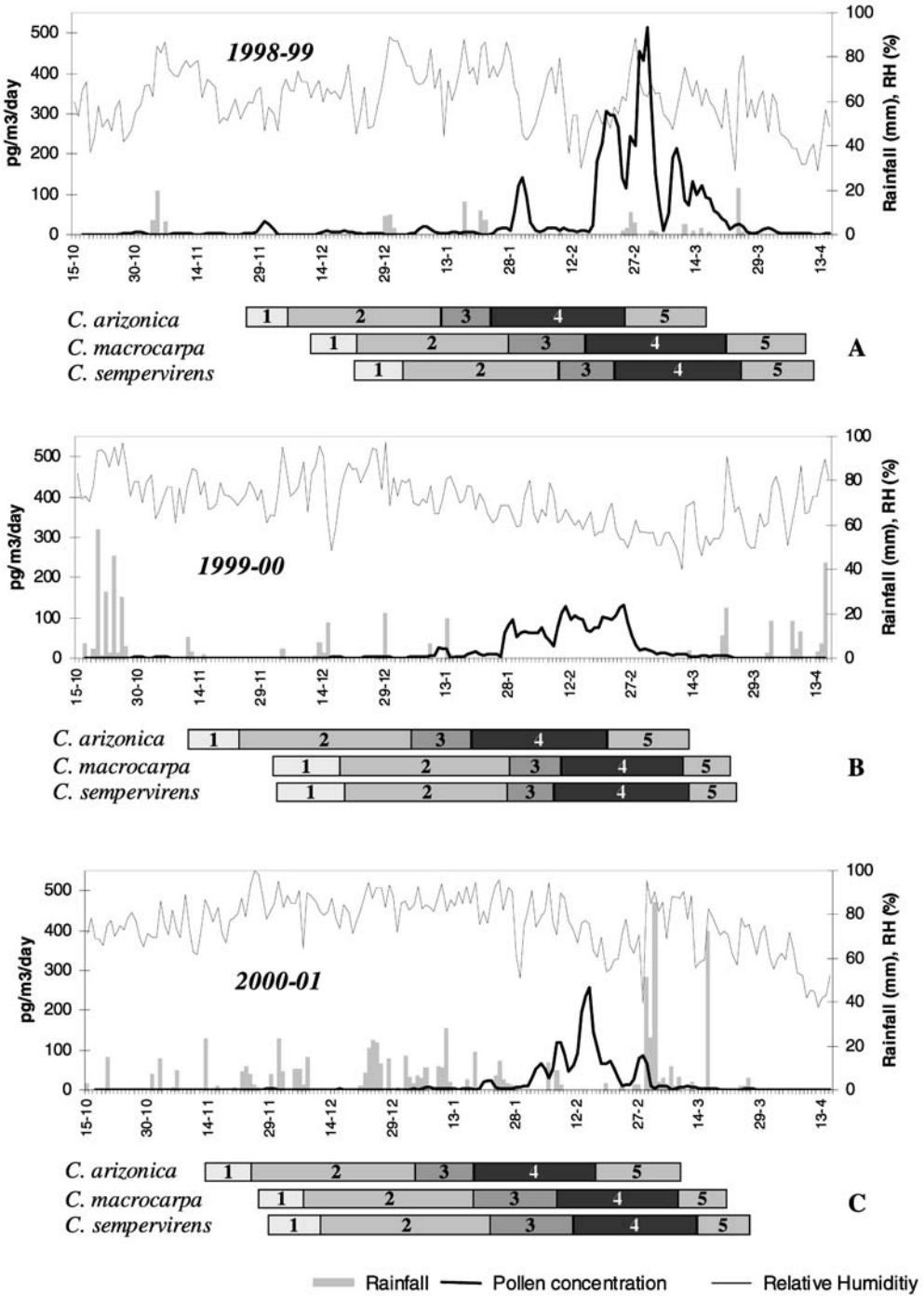

Fig. 3 Temporal distribution of the five male phenological phases in the three species of *Cupressus* studied during the winter-spring of 1998, 1999, 2000 and 2001. Cupressaceae airborne pollen, rainfall and relative humidity during the studied years. Phenophase 1 (bud differentiation) was the shortest phase, lasting from 11 to 17 days depending on the year. Phenophase 2 (immature flower) was generally the longest phase, lasting from 38 days in the first year to 42 days in the last year. Phenophase 3 (near to flowering) lasted 2–3 weeks. Full flowering (phenophase 4) lasted for approximately 1 month. The last phenophase (5, senescent period) seemed to vary substantially (2–3 weeks) between species and years

Temporal distribution of male phenological phases

Figure 3 shows the temporal distribution of male phenological phases over the 3 study years. In general, different pollen shedding periods were recorded for the 3 years. *C. arizonica* flowered from 20 to 23 days before the others. *C. macrocarpa* and *C. sempervirens* flowered almost simultaneously, although the former appeared to flower slightly earlier (except in the 2nd year). Temporal distribution of phenophases among the different species was delayed by approximately 2 weeks during the first year compared with the others. The length of each phenophase was approximately the same for the three studied species. Phenophase 1 (bud differentiation) was the shortest phase, lasting 11–17 days, depending on the year. Phenophase 2 (immature flower) was generally the longest phase, lasting from 38 days in the first year to 42 days in the last year. Phenophase 3 (near to flowering) lasted 2–3 weeks. Full flowering (phenophase 4) lasted approximately 1 month. The last phenophase (5, senes-

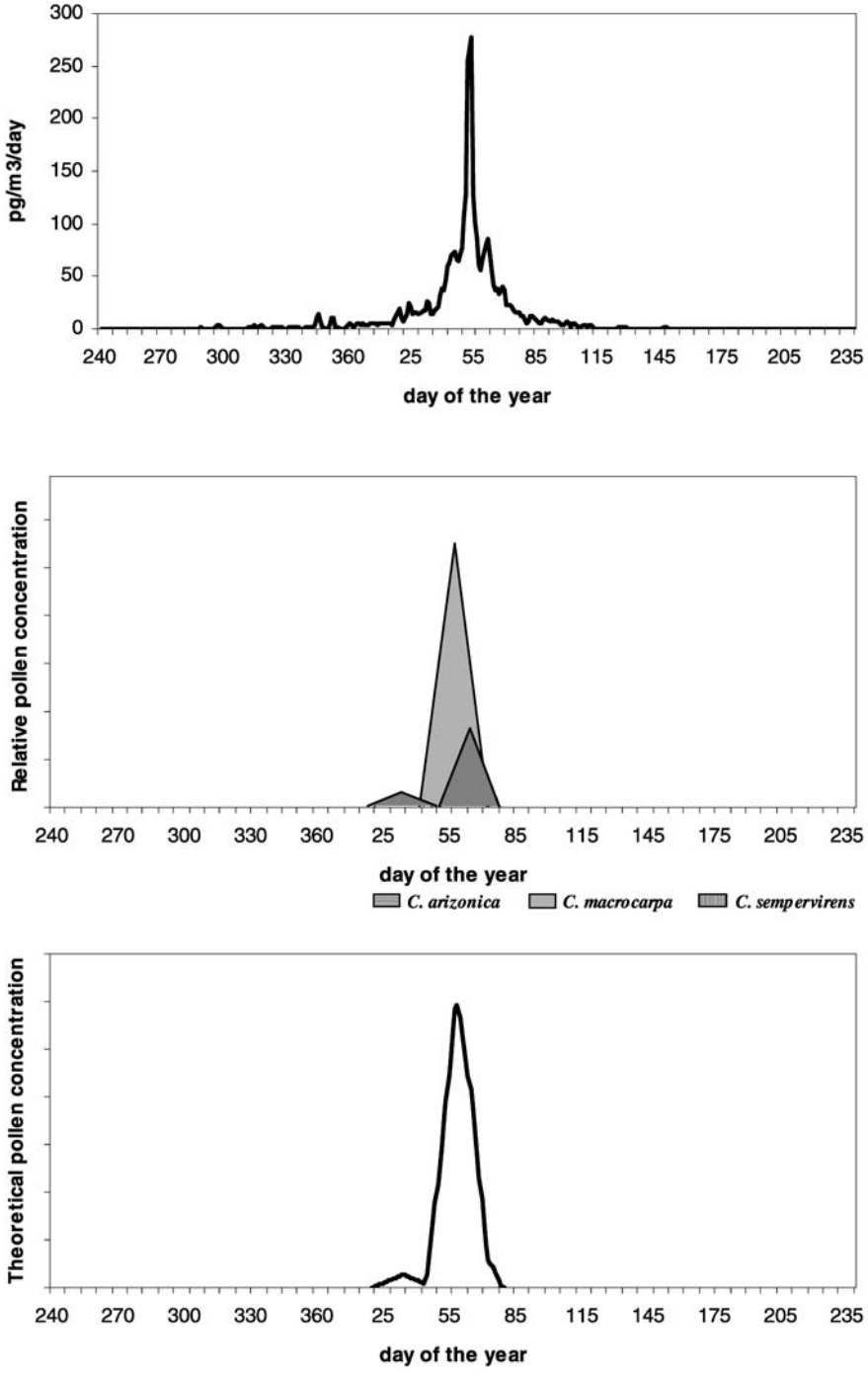

Fig. 4 a Three-day moving average pollen concentration from 1982 to 2000 in the city of Cordoba. Mean values were taken once the data had been aligned according to the maximum peak of concentration; **b** relative pollen emission of the three species; **c** theoretical model of *Cupressus* pollen emission

cent period) seemed to be very variable (2–3 weeks) between species and years.

C. arizonica trees produced a second pool of flowers a number of weeks (2–4 weeks) after the main flowering period (data not shown). This pool of flowers are halted at the close of phenophase 2 and remained at this stage during summer, autumn and winter. These flowers were joined by the normal pool of flowers set in early winter (phenophase 1 and 2 in Fig. 1). Both new and old flowers appeared to blossom simultaneously, and were at this time indistinguishable. This second set of flowers has not been observed in the other species of *Cupressus* and seems to be characteristic of *C. arizonica*. This special behaviour of *C. arizonica* has been observed in other parts of the Mediterranean, such as France and Italy (personal observations).

Relative pollen production per species

A total of 1,507 individuals were recorded in the study area: *C. sempervirens*, 74%; *C. macrocarpa*, 18%; and *C.*

arizonica, 8%. *C. sempervirens* was the most popular species, followed by *C. macrocarpa*. The least represented species was *C. arizonica*. Total pollen production per tree was 64,452 million in *C. sempervirens*, 122,951 million in *C. arizonica* and 1,141,075 million in *C. macrocarpa* (Hidalgo et al. 1999). Hence, the real percentage of potential pollen release was as follows: *C. sempervirens*, 18%; *C. macrocarpa*, 78%; and *C. arizonica*, 4%. More than three-quarters of pollen produced in the area can be attributed to *C. macrocarpa*, less than 20% to *C. sempervirens* and the amount of *C. arizonica* seems to be irrelevant when compared to the other species.

Correlation with airborne pollen

Figure 4a shows the 3-day moving average pollen concentration between 1982 and 2000 in the city of Cordoba. Cupressaceae airborne pollen showed a more or less symmetric pollen concentration curve. The main pollen-shedding season (95% of the total area of the curve) took place in the first 3 months of the year, coinciding with the end of winter and start of spring. The main peak occurred approximately between the end of February and the beginning of March.

Figure 3 shows the Cupressaceae pollen curve during the years studied. In general, the main pollen season corresponded to phenophase 4 of the three species. The pollen curve was strongly influenced by rainfall and RH. Several peaks were detected during the first year (1998–1999). The first peak, with a maximum of approximately 150 pollen grains/m^3, coincided with the first part of the full flowering phase (phenophase 4) of *C. arizonica*. Half of phenophase 4 of *C. arizonica* did not seem to be represented in the pollen curve although some rain (less than 3 l/m^3) and a long period of high RH occurred during this period. The last part of this phenophase was well represented in the pollen curve, coinciding with the second peak (more than 300 pollen grains maximum). Nevertheless, this phase overlapped with the first part of the full flowering phase of *C. macrocarpa* and the first part of the same phase of *C. sempervirens*. Rainfall washing was especially evident during the full flowering of *C. macrocarpa* and *C. sempervirens*. Nevertheless, a high peak (up to 500 pollen grains/m^3) appeared to coincide with full flowering in these species. No evident peaks were detected in the pollen curve after the end of phenophase 4 of *C. sempervirens*.

The second year (1999–2000) was characterized by the scarcity of Cupressaceae pollen in the atmosphere and high RH. Three main peaks occurred with concentrations between 100 and 150 pollen grains/m^3. The shape of the curve appeared to be highly influenced by RH. The first peak coincided with the full flowering phase (phenophase 4) of *C. macrocarpa*. The second and third peaks coincided with the same phase of *C. macrocarpa* and *C. sempervirens*. The amount of pollen present in the atmosphere after flowering of the last species (*C. sempervirens*) was insignificant.

The pollen curve was again strongly influenced by rainfall and RH in the third year (2000–2001). A high peak of 250 pollen grains (maximum) and four small peaks can be detected. The four first peaks coincided with the full flowering of *C. arizonica*. The high peak corresponds to the overlapped flowering of the three species, despite coinciding with the start of the flowering period of *C. macrocarpa*. The amount of Cupressaceae pollen in the atmosphere seems to be insignificant after the flowering of *C. sempervirens*.

It is clear that the minor contribution of *C. arizonica* to total airborne pollen coincided with the estimate reported in the pollen production study (only 4% of total potential *Cupressus* pollen). The main pollen concentration peak coincided with the main flowering period of *C. macrocarpa*, which accounted for 78% of potential pollen production. The flowering period of *C. sempervirens* overlapped with the flowering of *C. macrocarpa*, both species accounting for 96% of the potential pollen spread.

By considering the different potential emission and different flowering times, we can separate the pollen contribution of the three species. The theoretical relative pollen emission of each species is shown in Fig. 4b. The sum of the three curves in Fig. 4b produced the theoretical pollen curve proposed in Fig. 4c. A comparison of this model with the real concentration yielded a correlation coefficient of R^2=0.45.

Discussion

A new and complete description of the phenological stages of the male flower of *Cupressus* has been described. This methodology enables us to survey and quantify the evolution of male flowering in the different species of the genus *Cupressus*. It is a simple and easy method that can be used in other places. The phenology of species of the genus *Cupressus* different to those studied here could be charted using the same methodology. Knowledge of tree phenological laws is important in order to determine their different contribution to airborne pollen.

Cupressus species show virtually simultaneous male flowering with the pool of flowers of a tree population emitting pollen at the same time. This contrasts with the gradual blossoming typical of anemophyllous deciduous trees, most of which have male flowers arranged in catkins with a gradual mechanism of flower maturation. Nevertheless, the gradual mechanism of microsporophyllous maturation (from the bottom to the top of the flower) allows a long period of pollen shedding (approximately 4 weeks), comparable with the timing of catkin maturation. A similar mechanism is described for the microsporophylls of the male strobili of *Pinus roxburghii* (Khanduri and Sharma 2000), which do not dehisce simultaneously but instead dehisce from the broader apical to the narrower basal end.

Trees of different species of the genus *Cupressus* show a distinct male phenological behaviour. Pollen release in *C. arizonica* always takes place roughly 3 weeks earlier than in the other species. Similar findings were reported by Zerboni et al. (1991) for this species, which flowers before other Cupressaceae, such as *C. sempervirens* in Florence. Nevertheless the end of the period of pollen release of *C. arizonica* always overlaps with the start of pollen release of the other species, whereas the blossoming period is approximately the same in *C. macrocarpa* and *C. sempervirens* with only some days of difference. The conjunction of these three species led to continuous pollen shedding to the atmosphere, varying from 45 to 71 days in the city of Cordoba (Galán et al. 1998b).

The pattern of intra-species phases was approximately the same from year to year. The main inter-annual difference was in the start of the first phenophase. It is well known that both temperature and rainfall influence the reproductive phases of plants. In the case of Cupressaceae in Cordoba, regression analysis revealed that minimum temperature in October and rainfall in December are the main variables influencing the start of the pollen season (Galan et al. 1998a).

The secondary flowering recorded in *C. arizonica* does not seem to be directly related with a special climatic condition in our area since it also takes places in other regions of Europe. Unfortunately we have not found any phenological studies that describe *C. arizonica* in its natural range and currently have no information to enable an interpretation of this special behaviour. This plant may not be ideally suited to our climate, which is rather different than the conditions in its natural area. If this is the case, tree physiology may trigger flowering some months before, confusing the temperatures of the end of spring with those recorded at the beginning of autumn. This pool of flowers does not blossom due to the absence of cold temperatures. Flowering is triggered again at the start of autumn, just like in other species, and the flowers continue to develop reaching the same phase as the previous ones. Then, both new and old flowers develop simultaneously to flower at the same time.

Pollen shedding by the different species of *Cupressus* is positively correlated with Cupressaceae airborne pollen counts during the 3 study years. In most cases, rainfall (and associated moisture) accounts for the lack of coincidence between flowering and the airborne pollen curve. It is well known that the atmospheric pollen concentration fluctuates with weather changes. The usual effect of rainfall is to wash the particles in the atmosphere, at least when a heavy shower occurs. Some Cupressaceae species have hygroscopic mechanisms protecting their pollen from the rain. In *Juniperus*, cone scales interlock closely in damp weather and allow pollen release only in dry weather, when cone scales become separated (Gregory 1973). Anther dehiscence and pollen release are also affected by RH as described for *Pinus* in Khanduri and Sharma (2000). RH has the greatest negative effect on Cupressaceae airborne pollen (Galan et al. 1998b).

The first part of the Cupressaceae airborne pollen curve corresponds with the blossoming of *C. arizonica*; the rest of the curve overlaps with blossoming of *C. macrocarpa* and *C. sempervirens*. Taking into account the potential pollen production, the results confirm that the contribution of *C. arizonica* to airborne pollen is very minor in the city of Cordoba; the other species are responsible for most airborne pollen. *C. macrocarpa* accounts for 18% of total pollen from *Cupressus* trees but seems to be responsible for most airborne pollen. One striking feature is the low level of potential pollen production in comparison with the total number of *C. sempervirens* trees. This species is well represented in the city but their contribution to the airborne pollen is low (less than 20%) when compared with *C. macrocarpa*. The average pollen curve of Cupressaceae shows a more or less symmetrical shape despite being the result of the partial contribution of several species with a distinct phenology. Nevertheless, the curve shows a slight shift to the right, possibly due to the airborne pollen contribution of *C. sempervirens* trees, which overlap with the main peak produced by *C. macrocarpa*. Some small peaks before the main peak coincide with the main flowering period of *C. arizonica*. The proposed model accounts for a high percentage of the shape of the pollen concentration curve. This model is extremely useful for forecasting the amount of airborne Cupressaceae pollen in the city of Cordoba.

The use of internal traits, such as microsporogenesis stages, helps us to determine the phenological phases. Although it is impossible to survey the stage of microsporogenesis during phenological observations in the field, at least the phenophases described from this standpoint are fairly realistic. This fact is frequently ignored when studying the phenology of individuals. Knox (1984) estimated the timing of grass pollen development. However, information on other angiosperm and, more so gymnosperm pollen, is very scarce. The development of the sporangium in most conifers is very slow. In some species of *Pinus* or *Juniperus*, the winter is spent in the microspore mother-cell stage (Chamberlain 1965). After winter, meiosis and microspore maturation take place and pollen development concludes. Young buds become recognizable in *Cupressus* in late autumn and anthesis take places approximately 2 months later and still in winter. This means that meiosis is not interrupted during winter in *Cupressus*. The first phenophase is characterized by bud differentiation. The different tissues involved in microsporogenesis are developed and differentiated during this phase. This process does not take long time in comparison with the length of phenophase 2. After bud differentiation, sporogenous tissue develops and meiosis commences. Meiosis is a fast process that takes a little longer than normal mitosis. Early microspore maturation takes place after meiosis. Early maturation takes a long time in comparison with the other phases, as has been described for grasses (Knox 1984). Late maturation of microspores also takes several weeks (phase 3) and consists mainly of certain transformations

in the cytoplasm in addition to the increase in volume of the microspore. The maturation of the microspore/pollen seems to be the longest phase of microsporogenesis, as described by Knox (1984) for Poaceae. It is worth emphasizing that microspore/pollen maturation takes a long time despite the absence of mitosis during microsporogenesis.

There is no clear evidence of secondary raising of pollen in our study. This contrasts with the study performed by Keynan et al. (1989). In their study, the correlation between Cupressaceae airborne pollen and *C. sempervirens* flowering was not absolute. A substantial amount of Cupressaceae pollen was recorded after apparent flowering had ceased. These authors assumed that these pollen grains were raised secondarily. In these cases, pollen-monitoring stations offer a unique opportunity to detect this pollen in the atmosphere. In our study, the correlation between the three *Cupressus* species was almost absolute, the presence of non-corresponding pollen being attributable to a possible scattered flowering effect or to other Cupressaceae species whose contribution to airborne pollen was insignificant. Long-distance transport of *Juniperus ashei* pollen was shown by Rogers and Levetin (1998). In this study, Cupressaceae pollen was evaluated in significant concentration in an area where the source vegetation was not present. This is extremely important and might account for allergenic episodes when plants are apparently not emitting pollen. The contribution from upwind pollen sources might account for changes in the aerobiological records (Van der Water and Levetin (2001). There are no large stands of *Juniperus* or any other Cupressaceae far from our sampling point that could be potential sources of Cupressaceae pollen. Besides, most *Cupressus*-sensitive patients in Cordoba show seasonal symptoms, which only last the length of the standard Cupressaceae pollen season in the area (Guerra et al. 1996).

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