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Effects of environmental factors on pollen production in anemophilous woody species

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Abstract The aim of this study was to estimate the amount of pollen produced by anemophilous woody taxa with allergenic properties and with considerable contribution in the concentration of pollen in the air of a Mediterranean city (Thessaloniki, Greece). The taxa selected are *Corylus avellana*, *Cupressus sempervirens* var. *horizontalis* and var. *pyramidalis*, *Olea europaea* and *Platanus orientalis*; each was studied in more than one sampling stations differing in elevation, direction or both. *O. europaea* produced the highest number of pollen grains per flower ($1.3 \times 10^5 \pm 0.1 \times 10^5$) and *P. orientalis* the highest per inflorescence ($3.3 \times 10^6 \pm 0.2 \times 10^6$). At the level of crown, pollen grains produced were of the order of 10^9 per surface/volume unit for *O. europaea* and the two *C. sempervirens* varieties; for the other two taxa, they were of the order of 10^6. Pollen production was lower at higher elevation and northern direction and depended on the size of the floral unit sampled (flower for *O. europaea*, inflorescence for all other species): the bigger the floral unit, the more pollen it contained. Our results and reports from other areas, where *C. sempervirens* and *O. europaea* grow, show that these two Mediterranean species produce comparable amounts of pollen at the levels of inflorescence or flower, respectively, wherever they occur.

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Introduction

Plants produce pollen as part of their reproductive function. Anemophilous species, in particular, produce copious amounts of pollen so as to compensate for low pollination efficiency and ensure fertilisation. Pollen production estimates may serve in assessing and forecasting several parameters, related or not to reproduction. For instance, in forestry or agriculture, they can help in forecasting the future crop yield and the size of fruit and seed production (Allison 1990; Faegri and Iversen 1989; McKone 1990; Westgate et al. 2003). Pollen production is sensitive to environmental variability (Jablonski et al. 2002; LaDeau and Clark 2006; Rogers et al. 2006; Wan et al. 2002; Wayne et al. 2002; Ziska and Caulfield 2000); given this sensitivity, monitoring pollen from different responsive species may provide means for monitoring and evaluating local and/or global environmental changes. Moreover, the type and extent of the airborne and deposited pollen can provide information on present and past (more recent or remote) vegetation (Moore et al. 1991; Rogers 1993; Subba Reddi and Reddi 1986). Finally, as pollen inhalation from specific taxa induces respiratory allergy symptoms in sensitised individuals (Díaz de la Guardia et al. 2006; Gioulekas et al. 2004; Larese Filon et al. 2000), pollen production estimates can serve in forecasting the severity of respiratory allergies and alerting sensitised individuals.

There are estimates of pollen production for many species, primarily herbaceous; for woody plants, there exists information for representatives of the genera *Alnus*

(Moe 1998), *Betula* (Jato et al. 2007a), *Cedrus* (Khanduri and Sharma 2009), *Cupressus* (Hidalgo et al. 1999), *Olea* (Cuevas and Polito 2004; Ferrara et al. 2007; Tormo Molina et al. 1996), *Pinus* (Khanduri and Sharma 2002b; Ladeau and Clark 2006), *Platanus* (Tormo Molina et al. 1996), *Quercus* (Gomez-Casero et al. 2004; Jato et al. 2007b), *Taxus* (Allison 1990), and for several other species collectively studied (Mondal and Mandal 1998; Subba Reddi and Reddi 1986; Tormo Molina et al. 1996).

Several biotic and abiotic factors are found to influence pollen production. Among them, the meteorological factors, air temperature primarily, have profound impacts both at the macro- and the micro-environmental scales (Faegri and Iversen 1989; Moore et al. 1991). Despite this, the plasticity of pollen production under different environmental regimes has not been thoroughly studied so far; in most cases, species are studied in only one site and/or for one reproductive period. The levels at which sampling is conducted and pollen production is estimated are mainly those of the inflorescence, flower and anther (Allison 1990; Bera 1990; Beri and Anand 1971; Cruden 1977; de Vries 1971; Joppa et al. 1968; Moe 1998; Mondal and Mandal 1998; Spalik and Woodell 1994; Subba Reddi and Reddi 1986). For trees and shrubs, pollen production at higher levels, such as per crown size unit, per individual or per surface area, is only rarely assessed, as extrapolation of measurements to these levels is an arduous task (Moore et al. 1991; Rogers 1993).

Sampling methods used in the field and laboratory techniques applied for pollen isolation and yield estimation lack uniformity and some of them do not seem to be fully reliable (Moore et al. 1991). Also, the sampling size does not always seem adequate to deal with the magnitude of the variability observed. Given these, the reliability of estimations can become disputable (Moore et al. 1991) and so can the credibility of explanations for the differences reported among and within taxa, even from the same area. As Subba Reddi and Reddi (1986) argued, differences in estimations may be due to environmental or genetic factors, but they could also be the result of inadequacies of the methods used.

The aim of this study was to assess the pollen produced by four wind-pollinated woody species whose pollen is allergenic (Gioulekas et al. 2004) and which largely contribute to the total airborne pollen concentration of Thessaloniki, Greece (Damialis et al. 2007), and further elaborate pollen production estimates at different levels of analysis. To do so, we took into consideration the environmental and growth-trait variability. We also took into consideration the methodological weaknesses and tried to amend them so as to arrive at reliable results. There is information on pollen production, at one at least level of analysis, for two of the four species, i.e. for *Cupressus sempervirens* from Spain and for *Olea europaea* from Spain, Italy and California; therefore, an additional objective of our study was to compare the species' performance in geographically different areas.

Materials and methods

Study area and species

The study was conducted in the area of Thessaloniki (40°37′N, 22°57′E), the second largest city of Greece. Situated in the north of the country, the city has a mediterranean-type climate, characterised by warm and dry summers and wet and rather mild winters. The species selected for the study are *Corylus avellana* L., *Cupressus sempervirens* L. (two varieties, *C. sempervirens* var. *horizontalis* L. and *C. sempervirens* var. *pyramidalis* L.), *O. europaea* L. and *Platanus orientalis* L. They were selected because (a) of their considerable contribution to the total regional atmospheric pollen load (Damialis et al. 2007), (b) of the allergenic properties of their pollen (Gioulekas et al. 2004), and (c) they are representative species of different habitat types of the regional vegetation. Each taxon was studied in at least two stations differing in elevation, direction or both. Details for the 13 stations, where these taxa were studied, are given in Table 1.

Field work

This study was conducted over two consecutive years (2004–2005). To estimate pollen production, in each sampling station we selected ten healthy and mature individuals. These add up to a total of 130 individual plants for all species; samples were taken from the same individuals in both years of study. In addition, we took growth-trait measurements of the selected individuals. In particular, we measured (a) the perimeter of trunks (in m), (b) the height of crowns and individuals (in m) by use of the Blume-Leiss equipment (Matis 1994), (c) the crown diameter (in m), estimated as the average of two perpendicular to each other diameters of the crown at its widest part, and (d) the number of stems per individual (in *C. avellana* and *O. europaea*).

The floral unit that we used for the extraction of pollen was the flower for *O. europaea* and the inflorescence for all the other species. From each individual sampled, we collected four floral units, each from a north, south, east and west direction of the crown (Table 2). Floral units were collected just before flowering, so that their stamens (microsporophylls in *C. sempervirens*) were not visible yet; earlier studies (Damialis 2010) have helped us identify visual signs indicating when floral units, still closed, are

Table 1 Sampling stations for the five plant taxa examined

Taxon	Sampling stations	
	Location	Elevation, direction
Corylus avellana	Mt Hortiatis	900 m, S
		900 m, N
Cupressus sempervirens var. *horizontalis*	City centre	90 m, plateau
	Seich-Sou forest	430 m, plateau
		430 m, S
		430 m, N
Cupressus sempervirens var. *pyramidalis*	City centre	90 m, plateau
Olea europaea	City centre	0 m
	Sfendami, Pieria mountains	100 m, S
		100 m, N
	Plagiari, Thessaloniki suburbs	270 m, plateau
Platanus orientalis	City centre	0 m
	Mt Hortiatis	650 m, plateau

mature and ready to open. For each floral unit sampled, we measured length and maximum width. In each inflorescence sampled, we also counted the number of flowers that it contained.

In order to estimate pollen production at higher levels, we had to have a prior estimate of the production of floral units. In *C. sempervirens*, *O. europaea* and *C. avellana*, flowers/inflorescences appear at the outer part of the crown, at the end of branches (Hidalgo et al. 1999; Tormo Molina et al. 1996). Therefore, for each of these species, we measured on all selected individuals the number of inflorescences found within four sampling quadrats (25 cm × 25 cm), each from a north, south, east and west direction of the crown. In *P. orientalis*, inflorescences appear on the branches of the crown, for most of their length. Therefore, we measured the number of inflorescences in four, as before, cuboid sampling units (60 cm × 60 cm × *M* cm, where *M* is the distance between the trunk of the tree and the end of the selected branch). To estimate pollen production per crown volume unit, we took into consideration the geometrical shape of the crown; the shape was considered as approximating a cone for *C. sempervirens*, a spheroid for *O. europaea* and *C. avellana,* and an ovoid for *P. orientalis*.

Laboratory analysis

To extract pollen grains, each floral unit was put into a 10% KOH solution, which was boiled for 8 min to break up plant tissues and release pollen grains (Faegri and Iversen 1989; Moore et al. 1991). One of the problems encountered in pollen counting is the difficulty in bringing the pollen into uniform suspension. This is because pollen grains tend to clump due to the pollenkitt (or pollen coat) and the pollen surface charges (Shivanna and Rangaswamy 1992). To eliminate pollen clumping and avoid miscalculations of pollen production, we used glycerol, a bipolar solvent. Except for *P. orientalis*, we added 60% glycerol to a volume of 10 mL and we stained pollen grains in the suspension with safranin. Because of the much higher number of pollen grains per inflorescence and the more intense clumping in the case of *P. orientalis*, we added 85% glycerol to a final volume of 40 mL. With a CappAero

Table 2 Sampling units and levels of analysis for the estimation of pollen and floral unit production of the four species studied

Taxon	Production of pollen		Production of floral units	
	Sampling unit	Level of analysis	Sampling unit	Level of analysis
Corylus avellana	Inflorescence	Flower, inflorescence, m^2 of crown	Inflorescences in a crown quadrat (0.25 m × 0.25 m)	Flowers per inflorescence, inflorescences per m^2 of crown
Cupressus sempervirens[a]	Inflorescence	Flower, inflorescence, m^2 of crown	Inflorescences in a crown quadrat (0.25 m × 0.25 m)	Flowers per inflorescence, inflorescences per m^2 of crown
Olea europaea	Flower	Flower, m^2 of crown	Flowers in a crown quadrat (0.25 m × 0.25 m)	Flowers per crown m^2
Platanus orientalis	Inflorescence	Flower, inflorescence, m^3 of crown	Inflorescences in a crown cuboid [0.60 m × 0.60 m × length (m) of branch]	Flowers per inflorescence, inflorescences per m^3 of crown

[a] Under the terms flowers and inflorescences, we also include the sporophylls and the male cones of *C. sempervirens*, respectively

micropipette (Capp A/S, Odense, Denmark), we took two samples (10 μL each) per suspension, while stirring it vigorously to ensure homogenisation, and placed them on microscope slides under cover slips. Pollen grains on slides were counted at 100× magnification.

For the standardisation of the above methods, we conducted preliminary sampling and analysis during 2004. We checked for the minimum number of individuals (among 4, 8 and 10) and for the minimum number of floral units per individual (between 4 and 8) required for reliable assessments. We also checked for the concentration of the KOH solution (among 8, 10 and 12%), for the boiling time (among 5, 8 and 10 min), for the glycerol concentration (among 30, 40, 50, 60, 70, 80 and 90%), and for the number of sample replicates (among 2, 3 and 4). For these preliminary analyses, samples for each taxon were taken only from the lower station, except for *C. avellana*, for which samples were from two stations. To select the specific methodology adopted (sample size and laboratory techniques), we applied the criterion of low variability in pollen production estimates.

Estimation of pollen production

Pollen production *P*, estimated at different scales (Table 2), is described by the following equations:

(A) Pollen production per floral unit (flower or inflorescence; Table 1). The number of pollen grains per floral unit, P_{fu}, was estimated after the equation:

$$P_{\mathrm{fu}} = \frac{V_{\mathrm{su}}}{V_{\mathrm{sa}}}\overline{p}, \tag{1}$$

where V_{su} and V_{sa} are the volumes of the suspension (in mL) and of the sample taken (in μL), respectively, whereas $\overline{p}$ is the average over two replicates of the number of pollen grains in the sample.

(B) Pollen production per flower. The number of pollen grains per flower, P_{fl}, was estimated after the equation:

$$P_{fl} = \frac{P_{\mathrm{fu}}}{f}, \tag{2}$$

where f is the number of flowers per inflorescence (in *O. europaea*, $f = 1$).

(C) Pollen production per crown surface or crown volume unit.The number of pollen grains per surface unit (m^2) or volume unit (m^3) of crown, P_{cr}, was estimated after the equation:

$$P_{\mathrm{cr}} = P_{\mathrm{fu}}\frac{F_{\mathrm{su}}}{M}, \tag{3}$$

where P_{fu} is the number of pollen grains per floral unit as estimated above, F_{su} is the average number of floral units per crown sampling unit (quadrat or cuboid), and M is the area or the volume of the sampling unit.

(D) Pollen production per individual. The number of pollen grains per individual, P_{in}, was estimated after the equations:

$$P_{\mathrm{in}} = P_{\mathrm{cr}}S, \tag{4}$$

$$P_{\mathrm{in}} = P_{\mathrm{cr}}V, \tag{5}$$

where P_{cr} is the number of pollen grains per crown surface or volume unit, as described above, S is the total lateral surface area (in m^2) of the crown, estimated on the basis of the geometrical shape of each species' individuals, V is the total volume (in m^3) of the crown (estimated only for *P. orientalis*). As the geometrical shape of the individuals of *C. avellana* and *O. europaea* approximated a spheroid, of *C. sempervirens* a cone, and of *P. orientalis* an ovoid, the crown lateral surface of the first three species and the crown volume of *P. orientalis* were estimated after the following equations (Beyer 1984):

(a) Lateral surface for the spheroid crown-shape of *C. avellana* and *O. europaea*:

$$S = \frac{\pi d_m^2}{2} + \frac{2\pi d_m h_c}{\frac{\sqrt{h_c^2 - d_m^2}}{h_c}\sin\frac{\sqrt{h_c^2 - d_m^2}}{h_c}} \tag{6}$$

(b) Lateral surface for the cone crown-shape of *C. sempervirens*:

$$S = \frac{\pi d_m}{2}\sqrt{\frac{d_m^2}{4} + h_c^2} \tag{7}$$

(c) Volume for the ovoid crown-shape of *P. orientalis*:

$$V = \frac{\pi d_1 d_2 h_c}{6}, \tag{8}$$

where $\pi \approx 3.14$, d_{m} is the average crown diameter, d_1 and d_2 are two perpendicular diameters of the crown, at its widest part, and h_{c} is the crown height.

Data analysis

Pollen production data were analysed at different scales (Table 2). We checked for differences among species [ANOVA, Post hoc (Bonferroni test)], but also between sampling years, elevations and directions of the sampling stations. The numbers of pollen grains per floral unit, of flowers per inflorescence and of floral units per m^2 of crown were analysed separately for each species using analysis of covariance (ANCOVA); year, elevation and/or direction were the categorical predictors, whereas the morphological traits of inflorescences or individuals were the covariates. Full factorial analysis was used for the detection of differences among these factors. We also considered interactions between categorical predictors and covariates. We investigated for relationships between reproductive output and size of producing structures using the full dataset for each species (factorial regression) and we estimated R^2 and the regression equations for each of the five taxa examined. In all analyses, we examined both the raw data of pollen production and their logarithms. Residual analysis for each forecasting model provided an assessment of the remaining noise. On the basis of the error size and distribution, we selected raw values, as, in most cases, logarithms did not give higher values of R^2 and more normally distributed errors. All statistical analyses were carried out in Statistica 7.

Results

The standardisation procedure of the methods finally adopted showed the following: when the number of individuals was below 10, data variability was high; therefore, we sampled from ten individuals from each station. The number of floral units per individual (4–8) did not influence significantly the estimation of pollen production, neither did the height, at which they were located on the crown; for this reason, we chose the lower number of floral units for further research. Similarly, no significant differences were found regarding KOH concentrations and boiling times; in consequence, the lowest concentration and time examined were adopted. Regarding glycerol (30–90%), a high concentration was needed in order to have a uniform suspension, in the case of *P. orientalis*; as the suspension became too thick and could not be stirred vigorously beyond 85%, we selected the latter concentration for the samples of this species.

After applying the above standardised methodology, we found the per floral unit pollen production (flower for *O. europaea,* inflorescence for the other taxa) of the five woody taxa varying within a rather short range, from 10^5 to 10^6 grains. *P. orientalis* had the highest pollen and flower production per inflorescence; for both, it was followed by *C. avellana* (Table 3). At the level of flower, the highest pollen production was observed in *O. europaea* (Table 3).

At the level of crown, pollen production varied within the range 10^6–10^9 grains per surface or volume unit. At this scale of analysis, the highest reproductive output, both as floral units and pollen grains, was observed in the two *C. sempervirens* varieties (Table 3). Differences between the two varieties were detected only in the size of and the number of flowers per inflorescence, with *C. sempervirens* var. *horizontalis* producing more flowers (Table 3) and larger inflorescences (Table 4). Results regarding growth

Table 3 Mean number of reproductive units (±standard error), at different levels of analysis, over all stations and years of study for each taxon

Taxon	Floral unit production[a]			Pollen production		
	Flowers per inflorescence	Flowers per surface or volume unit of crown[b]	Inflorescences per surface or volume unit of crown[b]	Per flower	Per inflorescence	Per surface or volume unit of crown[b]
Corylus avellana	207.1 (±6.9)[d]	9.4×10^2 ($\pm 1.6 \times 10^2$)[e]	0.4×10^1 ($\pm 0.1 \times 10^1$)[e]	3.9×10^3 ($\pm 0.7 \times 10^3$)[e]	7.9×10^5 ($\pm 1.3 \times 10^5$)[d]	3.2×10^6 ($\pm 0.5 \times 10^6$)[e]
Cupressus sempervirens var. *horizontalis*	17.7 (±0.3)[e]	1.2×10^5 ($\pm 0.1 \times 10^5$)[c]	6.6×10^3 ($\pm 0.4 \times 10^3$)[c]	1.9×10^4 ($\pm 0.1 \times 10^4$)[d]	3.5×10^5 ($\pm 0.2 \times 10^5$)[d]	2.2×10^9 ($\pm 0.4 \times 10^9$)[c]
Cupressus sempervirens var. *pyramidalis*	16.6 (±0.4)[f]	1.2×10^5 ($\pm 0.1 \times 10^5$)[c]	7.3×10^3 ($\pm 0.4 \times 10^3$)[c]	2.0×10^4 ($\pm 0.3 \times 10^4$)[d]	3.5×10^5 ($\pm 0.5 \times 10^5$)[d]	2.5×10^9 ($\pm 0.4 \times 10^9$)[c]
Olea europaea	–	1.0×10^4 ($\pm 0.1 \times 10^4$)[d]	–	1.3×10^5 ($\pm 0.1 \times 10^5$)[c]	–	1.3×10^9 ($\pm 0.1 \times 10^9$)[d]
Platanus orientalis	272.3 (±13.9)[c]	1.1×10^5 ($\pm 0.1 \times 10^5$)[c]	3.9×10^2 ($\pm 0.3 \times 10^2$)[d]	8.2×10^3 ($\pm 1.5 \times 10^3$)[e]	3.3×10^6 ($\pm 0.2 \times 10^6$)[c]	3.3×10^6 ($\pm 0.2 \times 10^6$)[e]

[a] Floral units are flowers for *O. europaea* and inflorescences for all the other taxa studied

[b] The production estimate per volume unit (m^3) concerns only *P. orientalis*; the production estimate per surface unit (m^2) concerns all the other taxa studied

[c–f] Significant differences ($p < 0.05$) among taxa per column

Table 4 Mean values (±standard error) of growth traits of the floral units and the individuals sampled per taxon and station

Taxon	Station (elevation, direction)	Volume of floral unit (mm^3)	Growth traits of individuals			
			Trunk perimeter (cm)[a]	Number of stems	Plant height (m)	Crown size[b]
Corylus avellana	900 m, S	555.5 (±130.3)	14.9 (±1.9)	9.2 (±2.0)	2.4 (±0.2)	219.3 (±57.0)
	900 m, N	391.3 (±49.3)	17.1 (±1.7)	13.6 (±2.1)	6.1 (±0.9)	300.6 (±66.8)
Cupressus sempervirens var. *horizontalis*	90 m, plateau	186.6 (±24.1)	111.7 (±9.5)	–	14.3 (±1.2)	92.2 (±8.8)
	450 m, plateau	186.9 (±25.3)	61.5 (±4.3)	–	9.3 (±0.6)	36.3 (±5.0)
	450 m, S	197.0 (±10.6)	57.6 (±4.2)	–	10.9 (±0.8)	50.8 (±8.9)
	450 m, N	198.3 (±24.3)	51.8 (±2.2)	–	11.4 (±0.5)	42.3 (±5.0)
Cupressus sempervirens var. *pyramidalis*	90 m, plateau	171.4 (±21.1)	103.5 (±4.0)	–	15.3 (±0.9)	62.3 (±5.7)
Olea europaea	0 m	41.4 (±3.6)	52.4 (±1.8)	3.3 (±0.4)	8.4 (±0.7)	4741.7 (±3857.0)
	100 m, S	50.4 (±4.5)	50.1 (±3.4)	3.5 (±0.3)	5.1 (±0.2)	824.7 (±327.5)
	100 m, N	65.4 (±4.3)	33.8 (±1.8)	1.1 (±0.1)	2.7 (±0.1)	274.5 (±116.2)
	270 m, plateau	65.7 (±5.7)	44.9 (±2.0)	1.0 (±0.0)	2.7 (±0.1)	70.8 (±4.6)
Platanus orientalis	0 m	386.5 (±32.0)	194.4 (±21.2)	–	20.0 (±2.1)	1948.6 (±408.8)
	650 m, plateau	376.6 (±27.8)	90.8 (±11.1)	–	14.7 (±2.4)	189.2 (±35.3)

[a] Measurements of trunk perimeter correspond to the average perimeter of four stems per individual for *C. avellana*, and for as many stems present for *O. europaea*, when in the form of a shrub

[b] For *P. orientalis*, the crown size was estimated in terms of volume (m^3); for all the other taxa studied, it was estimated in terms of surface (m^2)

traits for all taxa from all stations are found in Table 4; the respective pollen and floral unit production at different scales of analysis are shown in Table 5.

We examined the effects of sampling year, elevation and direction on production of pollen grains and floral units and also the effect of the size of the floral unit on its pollen content (Table 6). Differences were observed in the amount of pollen produced per inflorescence between years, but not to the same direction for all taxa; pollen production was higher in 2005 for both *C. sempervirens* varieties, but it was lower for *P. orientalis*. The number of flowers per inflorescence did not present any significant difference between years, for any of the taxa examined.

Differences were also observed among elevations and directions. Populations at higher elevations or northern directions were associated with lower pollen and/or flower production (Table 6); for instance, *C. avellana* produced smaller amounts of pollen grains per floral unit and *C. sempervirens* var. *horizontalis* fewer flowers per inflorescence (Table 6). No difference was observed with elevation in *P. orientalis* (Table 6).

The amount of pollen produced was found to be related to the size of the floral unit, be it a flower or inflorescence (Fig. 1); in the case of inflorescences, size influenced also the number of flowers that they contained. The strongest flower–pollen relationship was observed in *C. sempervirens* var. *pyramidalis* (Fig. 1c) and the weakest in *O. europaea* (Fig. 1d).

At the level of surface or volume unit of crown, there were only a few differences between years and among stations. Such were the cases of *C. avellana* and *C. sempervirens*, with the highest pollen production being observed at the lowest elevation and at southern direction (Table 7). Differences in pollen production at the crown level were found to be affected by the growth traits of individuals; plant height and crown size were the most important, albeit having a significant effect only in two of the five possible cases.

Discussion

We found a large plasticity of the reproductive output of the woody plants examined associated with various extrinsic and intrinsic factors. Differences among sampling years as well as higher pollen production from lower stations and southern directions are reported for various species (Fotiou et al. 2010; Guardia and Belmonte 2004; Jato et al. 2007a; McKone 1990; Moe 1998). Plasticity in pollen production is largely manifested under different environmental factors (LaDeau and Clark 2006; Rogers et al. 2006; Wan et al. 2002; Wayne et al. 2002; Ziska and Caulfield 2000) and has been reported for herbaceous and woody species from different climatic zones: for *Alnus incana* (Moe 1998), *Betula alba* (Jato et al. 2007a), *Cedrus deodara* (Khanduri and Sharma 2002a), *Chionochloa pallens* (McKone 1990), *O. europaea* (Ferrara et al. 2007), *Parietaria judaica* (Fotiou et al. 2010; Guardia and Belmonte 2004), *Pinus roxburghii* (Khanduri and Sharma

Table 5 Mean pollen and flower/inflorescence production (±standard error) per floral unit and per surface or volume unit of crown, at different elevations and directions, for five taxa

Taxon	Station (elevation, direction)	Production per floral unit[a]			Production per surface or volume unit of crown[b]		
		Flowers per inflorescence	Pollen grains per flower	Pollen grains per inflorescence	Number of flowers	Number of inflorescences	Number of pollen grains
Corylus avellana	900 m, S	190.2 (±8.0)	5.6×10^3 ($\pm1.2 \times 10^3$)	1.1×10^6 ($\pm0.1 \times 10^6$)	5.4×10^2 ($\pm1.2 \times 10^2$)	0.3×10^1 ($\pm0.1 \times 10^1$)	3.2×10^6 ($\pm0.8 \times 10^6$)
	900 m, N	219.1 (±7.0)	2.7×10^3 ($\pm0.5 \times 10^3$)	5.7×10^5 ($\pm0.6 \times 10^5$)	1.2×10^3 ($\pm0.2 \times 10^3$)	0.5×10^1 ($\pm0.1 \times 10^1$)	3.1×10^6 ($\pm0.8 \times 10^6$)
Cupressus sempervirens var. *horizontalis*	90 m, plateau	19.9 (±0.3)	1.9×10^4 ($\pm0.1 \times 10^4$)	3.8×10^5 ($\pm0.2 \times 10^5$)	2.1×10^5 ($\pm0.2 \times 10^5$)	1.0×10^4 ($\pm0.1 \times 10^4$)	4.0×10^9 ($\pm0.5 \times 10^9$)
	450 m, plateau	16.3 (±0.3)	2.1×10^4 ($\pm0.3 \times 10^4$)	3.3×10^5 ($\pm0.3 \times 10^5$)	9.2×10^4 ($\pm0.9 \times 10^4$)	5.5×10^3 ($\pm0.5 \times 10^3$)	2.0×10^9 ($\pm0.3 \times 10^9$)
	450 m, S	17.5 (±0.3)	1.8×10^4 ($\pm0.2 \times 10^4$)	2.9×10^5 ($\pm0.2 \times 10^5$)	1.2×10^5 ($\pm0.1 \times 10^5$)	7.1×10^3 ($\pm0.3 \times 10^3$)	2.2×10^9 ($\pm0.3 \times 10^9$)
	450 m, N	17.0 (±0.3)	2.0×10^4 ($\pm0.2 \times 10^4$)	3.1×10^5 ($\pm0.2 \times 10^5$)	7.4×10^4 ($\pm1.2 \times 10^4$)	4.3×10^3 ($\pm0.6 \times 10^3$)	1.8×10^9 ($\pm0.3 \times 10^9$)
Cupressus sempervirens var. *pyramidalis*	90 m, plateau	16.6 (±0.4)	2.0×10^4 ($\pm0.3 \times 10^4$)	3.5×10^5 ($\pm0.5 \times 10^5$)	1.2×10^5 ($\pm0.1 \times 10^5$)	7.3×10^3 ($\pm0.4 \times 10^3$)	2.5×10^9 ($\pm0.4 \times 10^9$)
Olea europaea	0 m	–	1.2×10^5 ($\pm0.1 \times 10^5$)	–	1.3×10^4 ($\pm0.2 \times 10^4$)	–	1.6×10^9 ($\pm0.3 \times 10^9$)
	100 m, S	–	1.5×10^5 ($\pm0.1 \times 10^5$)	–	1.2×10^4 ($\pm0.1 \times 10^4$)	–	1.9×10^9 ($\pm0.2 \times 10^9$)
	100 m, N	–	1.4×10^5 ($\pm0.1 \times 10^5$)	–	6.3×10^3 ($\pm0.8 \times 10^3$)	–	8.4×10^8 ($\pm1.0 \times 10^8$)
	270 m, plateau	–	1.2×10^5 ($\pm0.1 \times 10^5$)	–	9.1×10^3 ($\pm1.1 \times 10^3$)	–	1.0×10^9 ($\pm0.2 \times 10^9$)
Platanus orientalis	0 m	281.7 (±8.7)	8.5×10^3 ($\pm2.1 \times 10^3$)	3.4×10^6 ($\pm0.2 \times 10^6$)	9.9×10^4 ($\pm1.1 \times 10^4$)	3.7×10^2 ($\pm0.3 \times 10^2$)	3.4×10^6 ($\pm0.3 \times 10^6$)
	650 m, plateau	265.5 (±10.1)	7.9×10^3 ($\pm1.6 \times 10^3$)	3.2×10^6 ($\pm0.2 \times 10^6$)	1.2×10^5 ($\pm0.2 \times 10^5$)	4.1×10^2 ($\pm0.4 \times 10^2$)	3.3×10^6 ($\pm0.2 \times 10^6$)

Mean pollen, flower and inflorescence production (±standard error) per surface or volume unit of crown are also given

[a] Floral units are flowers for *O. europaea* and inflorescences for all the other taxa studied

[b] Production was estimated per volume unit (m^3) only for *P. orientalis*; for all the other taxa, production was estimated per surface unit (m^2)

Table 6 Factorial analysis of covariance (ANCOVA) of pollen production data for the five taxa studied, at the level of floral unit

Source of variation	*C. avellana*[a]		*C. sempervirens* var. *horizontalis*		*C. sempervirens* var. *pyramidalis*		*O. europaea*[b]	*P. orientalis*	
	Pollen per inflorescence	Flowers per inflorescence	Pollen per inflorescence	Flowers per inflorescence	Pollen per Inflorescence	Flowers per inflorescence	Pollen per flower	Pollen per inflorescence	Flowers per inflorescence
Year			*** (2005)	ns	*** (2005)	* (2005)	ns	* (2004)	ns
Elevation			ns	*** (0 m)			* (100 m)	ns	ns
Direction	* (South)	*** (North)	** (Plateau)	*** (South)			** (Plateau)		
Floral unit volume	***	*	***	***	***	**	***	***	***
Year, floral unit volume			*	*	ns	ns	ns	ns	ns
Elevation, floral unit volume			ns	ns			***	ns	ns
Direction, floral unit volume	ns	ns	ns	***			**		

Pollen and flower production per inflorescence (dependent variables) were checked against effects of year, elevation and direction (categorical predictors) with floral unit volume being the covariate. The interaction of all factors was also examined (to the second-order degree interaction level). The significance level p is given in all cases (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, *ns* non-significant). In parentheses, the year, elevation or direction associated with the highest production are indicated (see Table 1 for description of the selected stations)

[a] *C. avellana* was studied only in 2005

[b] Floral units are flowers for *O. europaea* and inflorescences for all the other taxa studied

2002b), four *Quercus* species (Gomez-Casero et al. 2004), 28 grass, 19 shrub and 7 tree species (Mondal and Mandal 1998), another 38 Poaceae species (Prieto-Baena et al. 2003), etc. In our study, differences seem to be more pronounced in the production of flowers and inflorescences rather than of pollen, as also reported by Jablonski et al. (2002) and Spalik and Woodell (1994).

We found the amount of pollen produced to be related to the size of the floral unit sampled. This positive relationship, which holds true for all taxa studied, is also testified in several other woody and herbaceous species, such as *Fraxinus angustifolia* [Oleaceae (Tormo Molina et al. 1996)], *Juglans regia* [Juglandaceae (Tormo Molina et al. 1996)], *O. europaea* [Oleaceae (Tormo Molina et al. 1996)], *Pinus pinaster* [Pinaceae (Tormo Molina et al. 1996)], *Platanus hispanica* [Platanaceae (Tormo Molina et al. 1996)], *Populus nigra* [Salicaceae (Tormo Molina et al. 1996)], *Quercus rotundifolia* [Fagaceae (Tormo Molina et al. 1996)], *Ulmus minor* [Ulmaceae (Tormo Molina et al. 1996)], *Parietaria judaica* [Urticaceae (Fotiou et al. 2010)], *Secale cereale* [Poaceae (Sapra and Hughes 1975)], *Zea mays* [Poaceae (Vidal-Martínez et al. 2004)], various species and varieties of *Triticum* [Poaceae (Beri and Anand 1971; de Vries 1971, 1974)], etc.

The variability in pollen and flower production estimates was higher at levels than that of the inflorescence, even among individuals of the same station. Such variability largely depends on the growth traits of individuals such as height and trunk perimeter and crown size, as they are affected by the prevailing macro- and micro-environmental factors (Fumanal et al. 2007; Giantomasi et al. 2009; Levanič et al. 2009; Martín-Benito et al. 2008; Oliveira et al. 1994; Suzuki and Suzuki 2009).

Values of pollen production for the taxa that we examined did not deviate considerably from those reported in the past, at the low levels of analysis. For instance, in *O. europaea* from California, Cuevas and Polito (2004) found average pollen production per flower to be only slightly lower than our estimations ($9.2–9.6 \times 10^4$ compared to 1.3×10^5 pollen grains, respectively). For *O. europaea*, there are estimates of pollen production per anther from Spain (Tormo Molina et al. 1996) and Italy (Ferrara et al. 2007), of 1.0×10^5 and 8.0×10^4 pollen grains, respectively. Given that olive flowers have two stamens, the per flower pollen yields are twofold these values, very close to the ones that we estimated at this level. Similarly, for *Cupressus sempervirens*, Hidalgo et al. (1999) estimated pollen yield to be 3.7×10^5 compared to 3.5×10^5 in our study; these authors refer to pollen grains per flower, but from what they write, we understand that they refer to inflorescence, which was the floral unit that we also sampled. We can argue, therefore, that these two

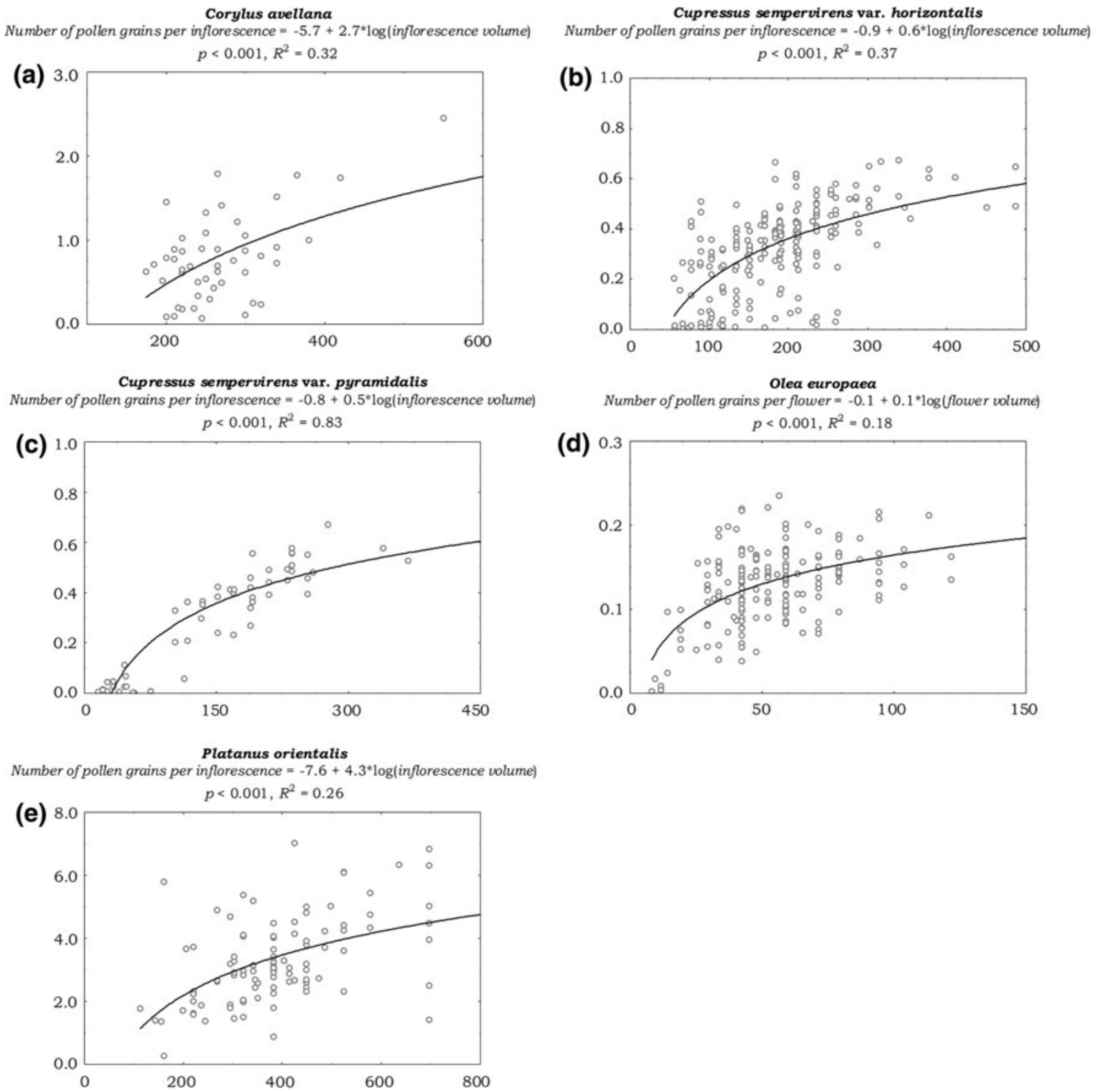

Fig. 1 Regression plots of the number of pollen grains per floral unit and floral unit volume, for five taxa. Pollen production (*Y* axis) is expressed as number of pollen grains ($\times 10^6$) per floral unit and floral unit volume (*X* axis) in mm^3. For each taxon, *p*, R^2, and the regression equation are given. The regression lines were fitted logarithmically. Note that different scales of pollen production are used in each plot

Mediterranean species produce very similar amounts of pollen at the level of flower, anywhere in the Mediterranean environment or in the climatically similar areas of the world, where they occur. Differences at higher levels, e.g., per unit of crown, per individual or per surface area, resulting from different environmental regimes and deriving from related effects on other attributes such as plant size, number and size of flowers, can be much larger. We selected the level of crown, instead of that of the individual, because extrapolations are made to a less extent and thus calculations are more reliable.

Estimates of pollen production show differences between entomophilous and anemophilous species with the latter producing more, but also between woody and herbaceous species, with the latter producing less (Mondal and Mandal 1998). Pollen production of entomophilous species is usually of the order of 10^3 or less, but sometimes it can reach 10^5–10^6 grains per flower, as in the case of *Bombax ceiba* (Bhattacharya et al. 1999), which can be attributed to its ambophilous nature. Similarly, in our study, for the ambophilous *O. europaea*, we estimated 1.3×10^5 grains per flower, which makes the species rank first among those examined. Such a high production could be the result of human intervention, under the effects of fertilisation, irrigation, pruning, or their combination, reported also for other species (Campbell and Halama 1993; Hall et al. 1982; Lau and Stephenson 1993). High numbers of pollen production might be due to the biannual

Table 7 Factorial analysis of covariance (ANCOVA) of the pollen production data for the five taxa studied, at the level of crown

Source of variation	*C. avellana*[a] Inflorescences per m^2 of crown	*C. sempervirens* var. *horizontalis* Inflorescences per m^2 of crown	*C. sempervirens* var. *pyramidalis* Inflorescences per m^2 of crown	*O. europaea* Flowers per m^2 of crown	*P. orientalis* Inflorescences per m^3 of crown
Year		ns	ns	ns	ns
Elevation		*** (90 m)		ns	ns
Direction	ns	* (South)		* (South)	
Trunk perimeter	*	**	ns	ns	ns
Plant height	ns	**	ns	ns	ns
Crown surface or volume	ns	**	*	ns	ns
Elevation, trunk perimeter	ns	ns		ns	ns
Elevation, plant height	ns	ns		ns	ns
Elevation, crown surface or volume	ns	ns		ns	ns
Exposure, trunk perimeter	**	*		ns	
Exposure, plant height	ns	**		ns	
Exposure, crown surface or volume	*	ns		ns	
Trunk perimeter, plant height	**	**	*	ns	ns
Trunk perimeter, crown surface or volume	**	**	ns	*	ns
Plant height, crown surface or volume	**	ns	**	ns	ns

Pollen and floral unit production per unit of crown surface or crown volume (dependent variables) were checked against effects of year, elevation and direction (categorical predictors) with trunk perimeter and height of individuals being the covariates. The interaction of all factors was also examined (to the second-order degree interaction level). The significance level p is given in all cases (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, *ns* non-significant). In parentheses, the year, elevation or direction associated with the highest production is indicated (see Table 1 for description of the selected stations)

[a] *C. avellana* was studied only in 2005

periodicity of *O. europaea*'s flowering; nevertheless, this does not seem to be the case, as there was no difference between the two years of study. Ferrara et al. (2007) suggest that higher pollen yields are the indirect effect of higher numbers of flowers produced; also, various researchers argue that pollen production per species at the level of anther does not vary significantly, because it is genetically fixed (Hidalgo et al. 1999; Subba Reddi and Reddi 1986). Though it is true that differences in pollen production are large when indirect effects are involved, our study shows that, at least in *O. europaea*, pollen yield varies considerably also at the level of flower suggesting a direct effect. This is in agreement with reports of marked differences of the amount of pollen produced at this level or at that of the anther (Davarynejad et al. 2008; de Vries 1974; Fotiou et al. 2010; Hill et al. 1985; Hyde and Williams 1946; Jato et al. 2007a; Palmer et al. 1978).

Various factors related to climate change influence directly or indirectly pollen production in woody anemophilous species. Depending on their population density in an area and their growth traits, differences in the number of pollen grains produced and hence emitted and circulating in the air may be very large. Information on the extent of pollen production and its plasticity is very important, particularly under the current climatic change, both from environmental and health perspectives.

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