Pollen biology in four Mediterranean Quercus species

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Potential pollen production, viability and germination were studied in the most important species of Quercus in the mountains of Córdoba to determine the contribution of each species to the total amount of airborne pollen. The results were compared over two consecutive years with different rainfall patterns. The viability of pollen grains was determined at anther opening, and during the pollination period, in order to determine potential pollination capacity. Results indicated that there were differences in the number of pollen grains produced by stamen in the four species. Equally, there were differences in the number of flowers among the species, being Q. suber the species with higher number of catkins groups and flowers per individual tree. Total pollen production per tree can be summarized in the following proportion: 1: 3: 3: 6 (Q. cocifera, Q. ilex ssp. ballota, Q. faginea and Q. suber).

Potential pollen viability was estimated using the Fluorochromatic Reaction (FCR) and a germination assay. The results have shown that Quercus potential pollen viability is high and declines slowly with time. Q. cocifera was the species with the highest percentage of germination, with Q. suber being the lowest.

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(Manuscript received 17 June 2002; accepted 10 October 2003)

Anemophily is based on an extremely indiscriminate and random dispersal mechanism; for that reason, anemophilous plants have developed an increase in pollen production in order to compensate for limited efficiency. Wind pollination is much less precise than biotic pollination, and a large number of pollen grains are required to ensure pollination. In general, the amount of pollen produced by wind-pollinated plants is higher than that from insect-pollinated plants, whether per stamen, per flower or per inflorescence (Proctor 1996).

Therefore, successful pollination depends in part on pollen production (Allison 1990). The capacity to produce pollen is under genetic and physiological control. Climate, in addition to influencing time of dehiscence and flower density, also influences pollen quantities produced (Stanley & Linskens 1974, Fornaciari et al. 1997, Galán et al. 2001). Pollen production per plant is determined by the number of pollen grains per anther, number of anthers per flower, number of flowers, and plant size. This data indicates the relative potential ability of each species to charge the ambient air with their pollen (Subba Reddi & Reddi 1986). Data concerning total pollen production per plant are valuable not only from an aerobiological and phenological (Hidalgo et al. 2003) but also from a forestry and agronomical viewpoint, since seed production depends on available airborne pollen (Campbell & Halama 1993, González et al. 1998, Galán et al. 2000, García-Mozo et al. 2002). On the other hand, fossil pollen records are important for reconstructing palaeobiomes. The quantitative relationships between the vegetation and pollen taxa are essential in reconstructing the past vegetation history (Carrión et al. 2001, Bunting 2002, Leira & Santos 2002). Data on the potential pollen production of different tree species has the potential to provide a more objective interpretation of fossil pollen data.

Pollen production varies widely among anemophilous species. A number of studies have addressed pollen production per plant in various species. Subba Reddi & Reddi (1986), for example, have calculated total production in certain angiosperms. Other studies have also provided extensive information on pollen grain production per anther, per flower, per inflorescence and per branch in both angiosperms (Pohl 1937, Tormo et al. 1996 and Prieto-Baena et al. 2003) and gymnosperms (Allison 1990, Hidalgo et al. 1999).

During pollen grain development within the anther, microsporogenesis may be affected by both genetic (Kaul 1988) and environmental stress (Neilson & Wullstein 1980, García-Mozo et al. 2001). Pollen is exposed to severe environmental factors during the dispersal phase that can affect pollen viability and germination capability (Stanley & Linskens, 1974). Viability mainly depends on relative atmospheric humidity at shedding and during pollen transport (Frankel & Galun 1977, Pacini 1990, Paolletti 1992). Although traditionally it has been considered that pollen remains viable longer at low relative humidity levels (Stanley & Linskens 1974) however, recent studies point out that pollen grains of different species need a high level of relative humidity to germinate (Corbet & Plumridge 1985,
MATERIAL AND METHODS

The city of Córdoba lies 123 m above sea level on the fertile lowland of the river Guadalquivir, between Sierra Morena, in the North, and Sierras Subbéticas, in the South (37°50’N and 4°45’W). The climate is Mediterranean, with some continentality. The area is characterized by temperate-cold winters and dry-hot summers, with an annual average temperature of 18°C, and an annual average rainfall of 600 mm.

The present study, over two consecutive years 2000 and 2001, took place in the foothills of the Sierra Morena, an area characterized by thermophilic vegetation with a reasonably well-preserved mixture of Quercus ilex ssp. ballota (holm oak), a medium-height (up to 25 m) evergreen; Quercus cocifera (Kermes oak), a small (1 – 2 m) evergreen shrub characterized by biennial acorn maturing; Quercus faginea (gall oak) a marcescent medium-height (up to 20 m) tree in forests, and a marcescent shrub in scrubland and Quercus suber (cork oak), a medium-height (up to 25 m) evergreen characterized by its cork-covered trunk. Flowers are arranged in catkins, which are clustered all along the branch. The typical androecium consists of a varying number of stamens (C.S.I.C. 1990).

A total of 120 mature and well-developed trees of the four species were selected for study; 30 trees per species.

Number of flowers per tree

In order to estimate the total number of flowers per tree, the following measurements were made: number of flowers per catkin; number of catkins per catkin group; number of catkin groups per square meter in the crown; radius of the crown.

In the four species studied, flowers are arranged in catkins clustered over the branches on the surface of the tree crown. The total number of flowers per catkin was counted by taking two branches (20 – 30 cm long) at random from the 30 trees selected for each Quercus species. The number of catkins per group was also counted.

The total number of catkin groups per m² of surface area in all selected individuals was counted by randomly placing a quadrant measuring 1 m² four times on each tree and extrapolating results to the total estimated surface area.

Estimation of total pollen grains per flower

In order to estimate total pollen grains per flower, three flowers were taken from 10 different catkins, on two trees for each selected species, using the count method proposed by Cruden (1977). Anthers, taken from closed flowers approaching anthesis, were crushed in 100 μl of distilled water. Total pollen was counted from this concentrate by depositing 10 μl on a slide. The count was repeated three times for each concentrate.

Distilled water was stained with fuchsin to facilitate the counting of pollen grains on the slide, for which a 4 × lens and a 10 × ocular were used. The total number of pollen grains per anther was multiplied by the number of anthers per flower to give the number of pollen grains per flower. Quercus species have a varying number of stamens. To estimate the average number of anthers, 30 flowers were chosen from different catkins and from different trees.

Estimation of total pollen grain production per tree

Total surface area was estimated for all selected individuals, considering the shape of the tree as a sphere. The radius of the crown was measured in each individual in order to calculate the area of the sphere by applying the sphere formula (4πr²). Using this result, the amount of flowers per tree was estimated by multiplying the total surface area by the number of catkin groups per m², by the number of catkins per group and by the number of flowers per catkin.

Total production per tree was estimated by multiplying: the average number of flowers per inflorescence by the number of catkins per group, then by the average number of groups per m², and by the total area of the individuals. The result was then multiplied by the average number of pollen grains per flower.
Statistical analysis

A paired-samples Student t-test was applied to compare means for the four *Quercus* species, using the data obtained in the various measurements.

Viability tests

Viability was estimated using two tests. Firstly, viability was measured in fresh pollen in 10 individuals for each of the four species, in a 23–30°C-temperature air environment, and 100% relative humidity. Temperature and relative humidity was chosen in the light of the results obtained by Vázquez et al. (1996) with *Q. ilex* ssp. *ballota* and *Q. suber*.

A varying number of catkins at anthesis were spread over a slide and pollen viability was estimated using the Fluorochromatic Reaction (Heslop-Harrison & Heslop-Harrison 1970), 18% in sucrose, in which viable pollen grains appear bright yellow under U.V. light. The total number of pollen grains on each slide was counted. The test was repeated twice for each individual. Viability was tested daily during the first week, and thereafter on a weekly basis for one month during the *Quercus* pollination period.

Moreover the actual percentage of germination was evaluated. To estimate pollen germination, the same number of individuals and catkins were analyzed. Again, catkins at anthesis were placed on slides coated with Heslop-Harrison semisolid medium (Heslop-Harrison & Heslop-Harrison 1986) in the same environmental conditions as the first assay (23–30°C temperature and 100% relative humidity). The percentage of germinability was estimated. In order to estimate the optimum period of time for germination completion, germinability was measured daily for one week. An optimum period of 48 hours was selected, as established by Jovanovic & Tucovic (1975).

RESULTS

Number of flowers per catkin

The average number of flowers per catkin obtained for *Q. ilex* ssp. *ballota* (25.4 ± 5.1) and *Q. suber* (22.6 ± 7.3) was very similar (Table I), but numbers were slightly lower for *Q. faginea* (19.8 ± 5.5) and *Q. coccifera* (15.3 ± 3.4). Comparison of the means for the four species using the *t*-test (Table III) revealed no significant differences in the number of flowers per catkin between *Q. ilex* ssp. *ballota* and *Q. suber*, although significant differences were found for the other species. Similar results were obtained for each study year.

Number of catkins

The average number of catkins per group were very similar for *Q. coccifera*: 4.3 and *Q. faginea*: 4, which differed from *Q. ilex* ssp. *ballota*: 3.5 and *Q. suber*: 2.5 (Table I); differences were noted both within each year and between the two study years. Comparison of the means for the four species using the *t*-test (Table III) revealed no significant differences between species with regard to the number of catkins per group.

However, the average number of catkins per m² differed both between species and between study years: *Q. coccifera* produced twice as many catkins per m² as the other species, between which no significant differences were recorded (Table III). A similar inter-species difference was found in...
the two study years, but all four species produced considerably more catkins per m² in 2001 (*Q. coccifera*: 70.8 in 2000 vs. 136.7 in 2001; *Q. ilex* ssp. *ballota* 45.9 vs. 70.3; *Q. suber*: 47.5 vs. 66.6; *Q. faginea* 48.3 vs. 56.3). Although significant differences were observed between *Q. coccifera* and the other species in 2000, no significant differences were found in the number of catkins per m² between the following pairs of species: *Q. ilex* ssp. *ballota* – *Q. faginea*, *Q. ilex* ssp. *ballota* – *Q. suber*, and *Q. suber* – *Q. faginea* (Table III). In 2001, no significant differences in the number of catkins per m² between *Q. ilex* ssp. *ballota* and *Q. suber* were observed, but differences between the other species were significant.

As a result, the number of catkins per tree differed among the four species, both within the same year and between study years. All species seemed to produce twice as many catkins in 2001 as in 2000, with the exception of *Q. faginea*, that produced the same amount each year (Table I). Moreover, the number of catkins per tree in *Q. suber* was the highest of the four species in both study years (45,248 catkins per tree in 2000, and 100,441 in 2001).

### Number of pollen grains per flower

The highest average number of pollen grains per stamen was obtained in *Q. coccifera* (7,355 in 2000 and 6,804 in 2001). Results were very similar for *Q. ilex* ssp. *ballota* and *Q. suber* both within the same year and between study years (around 3,500 pollen grains; Table II).

Similar results were obtained for the two study years, and also for *Q. coccifera*, *Q. ilex* ssp. *ballota* and *Q. suber*; however, results were quite different for *Q. faginea*, which produced much less pollen per stamen in 2001 (5,849 in 2000 vs. 676 in 2001).

Comparison of means values for the four species, using the *t*-test (Table III) revealed no significant differences in the number of pollen grains per stamen between *Q. ilex* ssp. *ballota* and *Q. suber*, either within the same year or between years. However, significant differences were found for the other two species.

### Total production per tree

The highest production of pollen grains per tree was recorded for *Q. suber*, and the lowest for *Q. coccifera* (Table II). Moreover, in 2001 *Q. suber* produced twice as many pollen grains per tree as in 2000 (24,046 × 10⁶ in 2000 and 55,045 × 10⁶ in 2001). Results for *Q. ilex* ssp. *ballota* and *Q. faginea* were very similar within the same year (11,919 × 10⁶ and 16,715 × 10⁶ respectively in 2000) and also between the two study years (24,500 × 10⁶ and 22,731 × 10⁶ respectively in 2001).

There were significant differences in the average number of pollen grains per tree between all species studied, except the *Q. ilex* ssp. *ballota* – *Q. faginea* and *Q. suber* – *Q. faginea* pairs in 2000 (Table III). In 2001 all species showed significant differences.

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**Table II. Area, number of catkins per tree and number of pollen grains per stamen, per flower, per catkin and per tree.**

<table>
<thead>
<tr>
<th></th>
<th>Area (m²)</th>
<th>Year 2000</th>
<th>Year 2001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catkins/tree</td>
<td>19.01</td>
<td>10.22</td>
<td>12.73</td>
</tr>
<tr>
<td>Pollen/stamen</td>
<td>1.015</td>
<td>0.0002</td>
<td>0.003</td>
</tr>
<tr>
<td>Pollen/flower</td>
<td>1.007</td>
<td>0.0001</td>
<td>0.003</td>
</tr>
<tr>
<td>Pollen/catkin</td>
<td>0.0005</td>
<td>0.0001</td>
<td>0.003</td>
</tr>
<tr>
<td>Pollen/tree (× 10⁶)</td>
<td>5.991</td>
<td>1.040</td>
<td>1.396</td>
</tr>
</tbody>
</table>

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Viability

The highest percentage of viable pollen grains on the first day of the measurement was obtained for Q. faginea (90%, Fig. 1 D) followed by Q. suber (83%, Fig. 1 A) and Q. ilex ssp. ballota (Fig. 1 B). The lowest percentage was obtained from Q. coccifera (69%, Fig. 1 C). However, these percentages decreased slightly after seven days of measurement: Q. suber (72%, Fig. 1 A), Q. faginea (69%, Fig. 1 D), Q. ilex ssp. ballota (61%, Fig. 1 B) and Q. coccifera (60%, Fig. 1 C).

The percentage of viable grains declined with time (Fig. 2); by the end of the month, the results were as follows: Q. suber (57%, Fig. 2 A), Q. faginea (50%, Fig. 2 D), Q. coccifera (48%, Fig. 2 C) and Q. ilex ssp. ballota (40%, Fig. 2 B).

Q. coccifera showed the highest percentage of germinability after 48h, with an average of 34.91% (Fig. 3), followed by Q. ilex ssp. ballota (29.73%), Q. faginea (26.02%) and Q. suber (18.12%).

DISCUSSION

From an aerobiological standpoint, data on total pollen production per plant, supported by knowledge of the coverage of the various species in a given area, could enable us to determine the partial contribution of each species to the total amount of pollen in the atmosphere (Tormo et al. 1996, Hidalgo et al. 2003, Prieto-Baena et al. 2003). Moreover, in recent years some researchers have demonstrated a relationship between the amount of airborne pollen and seed production (Allison 1990, Campbell & Halama 1993, Candau et al. 1998, Galán et al. 2000, García-Mozo et al. 2002).

As Tormo et al. (1996) have reported for anemophilous tree species, there is a constant value for pollen production between species. These species show a tendency to compensate for sexual characteristics (e.g. number of pollen grains per stamen, inflorescences per tree, etc.) by increasing some of them or reducing others, so that the resulting product generally lies within defined margins. In our work, differences have been observed between species within the same year; the evergreen shrub Q. coccifera produced the highest amount of pollen per stamen and a higher number of groups of inflorescences per m² than the other species. These results would suggest that this is a method of compensating for the smaller size of pollen grains, lower individual height and smaller crown diameter or size in this species.

Total pollen production per tree was higher for Q. suber than for the other species studied. This was to be expected, since total pollen-grain production per tree correlates positively with tree-crown diameter. There were differences in pollen production per tree between study years, the main difference was found for flower density (number of flowers per tree). Pollen production calculated here for Q. ilex ssp. ballota was over one hundred times lower than that reported for the same species by Tormo et al. (1996), probably because the trees studied in this area were smaller. However, the number of pollen grains per stamen was similar to that reported by those authors. This was to be expected, since the number appears to be under genetic and physiological control (Subba Reddi & Reddi 1986, Stanley & Linskens 1974). For this reason, the number of pollen grains per stamen remained fairly constant for each species between the two study years. Nevertheless, the results obtained for Q. faginea in 2001 were lower than in 2000. To account for these results, further research and data verification are required. This was the only deciduous species studied, and it may be that deciduous species are more sensitive to changes in the weather.

Effective pollination in anemophilous species has been suggested to depend on pollen shedding, which is related to diurnal patterns of temperature, relative humidity, and probably micro-environmental electrostatic conditions (Jones & Little 1983), as well as pollen production (Allison 1990). Therefore the higher pollen production in 2001 would provide a greater guarantee of wind pollination than in 2000.

Pollen production reportedly depends on rainfall and temperature over the period prior to flowering (Fornaciari et al. 1997, Galán et al. 2001). The average temperature in the months prior to flowering was very similar in both study years, but rainfall was higher in 2001 (Fig. 4). In 2001, the number of flowers per tree was higher than in 2000; this may be a result of the rainfall during the months prior to flowering. These results suggest that, in view of the wind intensity, humidity, rainfall, and temperature recorded in 2001, the number of pollen grains released into the air was greater than in 2000 since these parameters also influence pollen dispersal (Galán et al. 1998).

The amount of pollen released by individual members of each species can be summarized approximately in the following proportions: 1:3:3:6 (Q. coccifera: Q. ilex ssp.
ballota: Q. faginea: Q. suber). This represents the relative contribution of each species to airborne pollen in a given area, assuming the same number of individuals for each species. It would thus be useful to determine plant density per unit surface area in real zones, in order to estimate the actual contribution of those species.

Effective pollination depends not only on pollen production but also on pollen viability. Compatible and receptive stigmas are fundamental requisites for pollination. Stigma receptivity lasts from a few hours up to one month...
depending on the species (Frankel & Galun 1977). In this study, pollen viability in vitro decreased, over a 30-day period, from an average 80\% to 50\%. Nevertheless, differences in viability were detected between the species studied. *Quercus faginea* showed the highest percentage viability on the first day of measurement, and *Q. coccifera* continued to show the lowest viability, while the highest results were recorded for *Q. suber*. The steepest fall in viability occurred in all species between day 1 and day 7; the greatest difference in viability was recorded on the first day of measurement in all species except *Q. ilex ssp. ballota*. Viability did not vary greatly during the remainder of the pollination period. Pollen in some anemophilous trees, pines and palm trees, can be stored in viable condition for a year or more under natural conditions (Frankel & Galun 1977). Brewbaker (1967) observed, as a general trend, that trinucleate pollen loses its viability much faster than binucleate pollen. Pollen of the *Quercus* genus has been reported as binucleate (Stairs 1964). These findings would account for the longevity observed here in *Quercus* species pollen.

After 48 hours, *Quercus suber* pollen grains were found to germinate rapidly, usually within a few hours after pollination. In the present study, this species displayed the lowest percentage of germination for the four species studied. The highest was found for *Q. coccifera*. The result from FCR tests suggest that although potential capability of pollen grains to germinate is high, *Q. suber* displays a high percentage of viability over the first 48 hours, but it is the species with the lowest real range of pollen germination. These differences can be due to different causes: 1) The germination medium being optimal; 2) Some of the living grains under microscope examination lack the capability of pollen tubes growth; 3) Pollen grains that died a few minutes earlier remained apparently viable under UV light.

The data resulting from the FCR test were higher than those of the germination test, and this fact coincides with those found by Pickert (1988) in *Arabidopsis thaliana* L. This result shows the limitations of FCR test versus the germination of pollen in vitro used as viability assay. Pollen grain viability tested by FCR give us the potential capability of pollen to germinate, although the actual number of pollen grains that germinate is lower.

**CONCLUSIONS**

Different pollen production within the same year was observed in the three species studied. *Quercus coccifera* produced the highest amount of pollen per anther and catkin groups per m\(^2\) of tree crown. The total pollen production per tree was different for each *Quercus* species in Córdoba. The lowest value was obtained for *Quercus coccifera*, although a higher density of flowers was observed resulting in a similar amount of pollen production per tree. Several differences were found regarding the potential pollen production per tree between both years of study (2000–2001), largely due to a higher floral density per tree during 2001.

The relative contribution of each species to total airborne pollen quantity assuming the same number of trees per unit area for each *Quercus* species could be summarized by the following proportions, 1:3:3:6 (*Q. coccifera*: *Q. ilex ssp. ballota*: *Q. faginea*: *Q. suber*). Although the potential capability of pollen grains to germinate is high, *Q. suber*
displays a high percentage of viability over the first 48 hours, but it is the species with the lowest real range of germination. *Quercus coccifera* has the lowest viability index but a higher level of pollen germination.

**ACKNOWLEDGEMENTS**

The authors are grateful to the Spanish DGICYT for financial support granted through Project PB-96-0513. They wish to thank to Prof. Dr. J. L. Ubera of the University of Córdoba for his valuable help in the viability analysis.

**REFERENCES**


**Grana 43 (2004)**