Pollination Dynamics in *Vitis vinifera* L.

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Abstract: *Vitis vinifera* pollen dispersal was studied using volumetric air samplers situated within a vineyard to measure airborne pollen concentration. The pollen dispersal period of Cabernet Sauvignon was seven days, reaching daily mean concentrations of about 90 grains/m^3, with peak pollen dispersal near midday. Daily and hourly variations in concentration were positively correlated with temperature but negatively with relative humidity. Concentration was greatest within 1.5 m of the plants and up to 1 m aboveground, after which it diminished.

Key words: *Vitis*, phenology, pollen, pollination

While in most grapevine cultivars autogamy is sufficient to guarantee production (Heazlewood and Wilson 2004), many cultivars require cross pollination to reach full maturity (Chkhartishvili et al. 2006). Other cultivars are self-sterile and when the flowers are isolated, fruit maturation is inferior to that obtained during free pollination, as in Cardinal (Sharples et al. 1965), and certain cultivars and species with hermaphrodite flowers produce inaperturate pollen grains incapable of germinating (Lombardo et al. 1978). In all these instances, the level of pollination of the cultivar must be taken into account as it determines yield. Even in cultivars that produce seedless grapes, pollination is essential for normal fruit development, so that a high percentage of small fruit is produced when pollination is artificially reduced, as in Bolgar (Tsolova 1995).

Grapevine pollination is facilitated by wind or by insects. Insect activity has been described in Cardinal, for which installation of beehives did not increase the number of fruit, but did reduce the percentage of seedless grapes (Sharples et al. 1965).

The dispersal of grapevine pollen in winegrowing areas has been examined extensively since pollen concentrations were first analyzed in the atmosphere around Montpellier (Cour et al. 1972). However, most of these studies were focused on finding links between annual pollen concentrations and grapevine yield and on how environmental variables affect regional pollen dispersal (Cristofolini and Gottardini 2000, Cunha et al. 2003). In these, the samples were collected on the roofs of buildings located in vineyard areas, using mainly Cour-type samplers that register weekly pollen concentrations.

There is now renewed interest in the study of *Vitis* pollen dispersion following the first field tests of transgenic grapevines at various sites worldwide, resulting from the need to scientifically justify possible cross-pollination with other cultivars in the same zone. A pilot study on transgenic plants of the Dornfelder cultivar monitored pollen deposition at distances of 5 to 150 m from the pollen donor and determined the rate of outcrossing by sampling seeds of selected grapevines at different distances (Harst et al. 2009a, 2009b). This study aims to extend the knowledge of the dynamics of anemophilous pollination in the grapevine through the examination of variations in airborne pollen concentration, using volumetric air samplers situated within the vineyard, measured during the period of pollination and at different times of day and different sites within the vineyard.

Materials and Methods

The research was carried out at a vineyard for the cultivation of grapevine by irrigation in Zafra (Badajoz, southwest Spain). The vines were planted in rows with a space of 1.5 m between each vine, with each row 3 m apart. Cabernet Sauvignon is the main cultivar, although there is a plot in which nine other black grape cultivars are grown: Alphonse Lavallée, Cinsaut, Garnacha, Garnacha Tintorera, Mazuelo, Merlot, Morisca Real, Syrah, and Tempranillo (Figure 1). All the cultivars were grafted onto American rootstocks.

The pattern of pollen concentrations in the atmosphere at the vineyard was measured with a Burkard aerobiological sampler (Burkard Manufacturing, Richmansworth, UK) (Hirst 1952). It was installed on 16 May 2001 in the middle of the Cabernet Sauvignon vineyard (Figure 1) at a height of 1.5 m and was taken down on 5 June. The samples were

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analyzed under a microscope at a magnification factor of 400 with four longitudinal scans over the slide to obtain the pollen grapevine concentrations for the whole day and for each hour (Tormo et al. 1996). The mean and confidence interval for each hour was calculated to find the average concentration per hour. Daily meteorological data were obtained from the Llerena weather station, situated 35 km southeast of the vineyard, the nearest meteorological station that monitored hourly meteorological data in 2001. As daily and hourly pollen concentration data were not normally distributed (Shapiro–Wilk, W = 0.704, $p = 0.00006$, and W = 0.792, $p = 0.0002$, respectively), Spearman correlations were used to check the effects of daily and hourly mean temperature, relative humidity, wind speed, and wind direction on the airborne pollen concentration; values of $p < 0.05$ were considered significant. No rain was recorded during the study period.

To monitor Cabernet Sauvignon pollen grain dispersal at the vineyard, three analyses were made using portable Burkard samplers, which have the same trapping efficiency and sample analysis as that of fixed samplers. Sampling took place between the sunlight hours of 1600 and 1830. Since the purpose of this research was to monitor changes in concentrations as influenced by distance and location of pollen sources, it was necessary to consider the relevance of other unrelated factors, such as vineyard topography and microclimate. Thus, a pollen type was chosen whose concentrations in the vineyard were not influenced by the location of the sampler with respect to the site of the pollen source, and which would therefore show variations that were due only to those particular factors mentioned. Thus, pollen from olive trees, which were abundant in the area but which were no closer than 500 meters from the vineyard, was also monitored.

The first trial consisted of measuring the influence of height on the dispersal of pollen grains. The samplers were placed for 30 min at four different heights in the center of the Cabernet Sauvignon vineyard, between the vineyard rows. Grapevine and olive pollen concentrations were recorded at ground level, and then at 0.5 m, 1 m, and 2 m aboveground. These samplings took place on 30 May 2001.

To measure the horizontal dispersion of grapevine pollen, three locations were selected on the edges of the Cabernet Sauvignon vineyard: 1.5 m, 3 m, and 4.5 m from the vine rows. Fourteen samples of 30 min each were taken (6, 4, and 4, respectively) on 29 and 30 May, recording the mean concentrations of grapevine and olive pollen in each location. The analysis of these findings was made by calculating the regression line for the three positions of the concentrations from each pollen source.

To evaluate the actual effects of distance on concentration in the vineyard, the pollen concentrations in the 10 cultivars were monitored on 22 May, when only four cultivars had more than 10% of inflorescences with some open flowers: Cinsaut (47.5%), Garnacha (85.0%), Garnacha Tintorera (75.0%), and Merlot (22.5%). This data was obtained by counting 40 inflorescences in each cultivar. The concentration of grapevine and olive pollen between two rows of vines of the same cultivar was calculated by sampling for 30 min. These data were subjected to a Student's t-test to measure sample homogeneity. Pearson correlations between grapevine and olive concentrations were used to check whether the variations in grapevine concentrations were due to the phenological condition of the different cultivars or to variations in specific conditions. Values of $p < 0.05$ were considered significant.

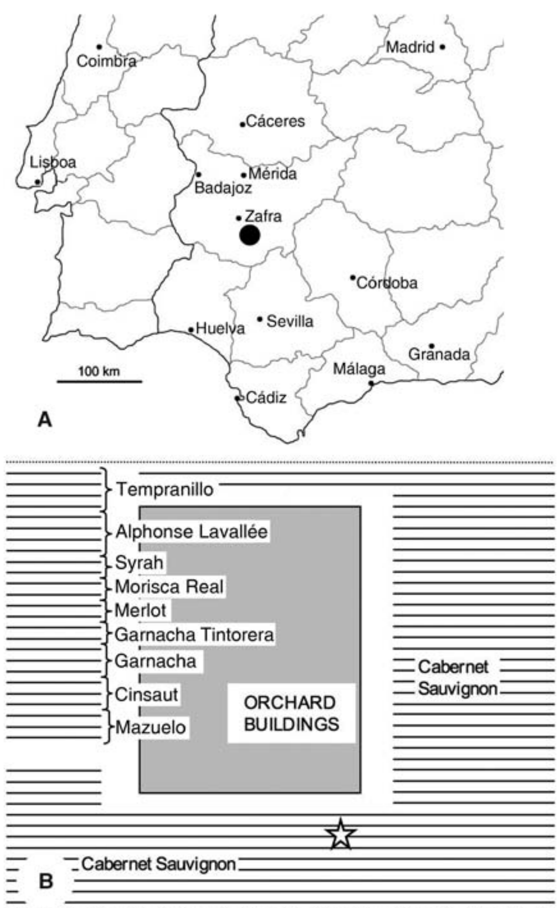

Figure 1 Location and description of the zone studied. Location of the vineyard (black circle) on the map of the southwestern Iberian Peninsula (**A**). Plan of the central zone of the vineyard indicating position of the aerobiological sampler (star) (**B**).

Results and Discussion

Maximum daily concentrations of grapevine pollen at the vineyard occurred between 26 May and 1 June, before and after which there were fewer than 10 grains/m^3 (Figure 2). With Cabernet Sauvignon predominant in the vineyard, it can be concluded that it shows a period of pollination that lasted only seven days, thus falling within the range of the mean date of flowering reported for Cabernet Sauvignon in Bordeaux (23 May to 27 June) (Jones and Davis 2000). This period is shorter than that recorded in eight other cultivars in Turkey that showed periods of between 13 and 17 days (Kelen and Demirtas 2003). The duration of the flower opening period is temperature-dependent, as high temperatures shorten the length of the period (Staudt 1999).

During the maximum pollination period for Cabernet Sauvignon, the mean concentration was 62.7 grains/m^3 with a peak of 87.5 grains/m^3 recorded on 29 May. These concentrations are low for an anemophilous cultivar, which can be seen if this peak is compared with that of olive pollen on the same date (170.0 grains/m^3). This contrast is consistent with observations that the dispersal of grapevine pollen is not extensive when compared to other anemophilous crops (Ribeiro et al. 2005). Results here are similar to those recorded using a volumetric sampler situated on the roof of a building at 10 m height to record maximum concentrations of between 41.5 and 197.5 grains/m^3 over five years, with total annual concentrations of between 325.5 and 755.5 grains/m^3 (Cristofolini and Gottardini 2000), which is consistent with the 492.2 grains/m^3 in our study.

During the study, daily mean temperatures ranged from 15.1 to 28.5°C; relative humidity, from 29.3 to 79.4%; daily wind speed, from 2.8 to 10.4 km/hr; wind direction, from 60° to 253° (ENE to WSW); and no rain was recorded (Figure 2). Temperatures showed a significant correlation with daily pollen concentration (r = 0.632, $p = 0.0037$). Similar conclusions were obtained by others who stated that high temperature and high light stimulated flowering (Ribeiro et al. 2005, Vasconcelos et al. 2009). Relative humidity affected pollen concentration negatively (r = -0.827, $p = 0.00001$). Wind speed (r = 0.255, $p = 0.292$) and wind direction (r = -0.198, $p = 0.416$) had no significant effect on pollen concentration.

Pollination levels of Cabernet Sauvignon were at their minimum between 2300 and 0700 hr, and at their maximum at midday (Figure 3). These data show a pattern that follows the flower openings of Müller-Thurgau and Blauer Spätburgunder cultivars, which started opening ~0500 hr, reached a maximum between 0700 and 0900 hr, and all the flowers were opened ~1200 hr (Staudt (1999). Hourly concentrations were affected by meteorological factors in the same way as daily concentrations: positively by temperature (r = 0.673, $p = 0.0003$) and negatively by relative humidity (r = -0.664, $p = 0.0004$). Wind speed (r = 0.366, $p = 0.078$) and wind direction (r = 0.196, $p = 0.358$) had no significant effects on pollen concentrations.

The influence of sampling height on the concentration of *Vitis* and *Olea* pollen was determined (Figure 4). The concentrations of olive pollen were greatest up to 0.5 m aboveground and reduced gradually as the sampling height was raised, possibly as a consequence of the reduced wind speed at samplers close to the ground. For Cabernet Sauvignon pollen, the concentrations were ~200 grains/m^3 at 1 m aboveground, highlighting the influence of proximity to the pollen sources. At 2 m, there was a 10-fold reduction in concentration, to 20 grains/m^3, demonstrating the limited vertical dispersion of the pollen of this cultivar, since the concentration reduces rapidly above the vine.

Pollen concentrations at different horizontal distances from the edge of the Cabernet Sauvignon vineyard were determined (Figure 5). *Olea* concentrations remained steady, but *Vitis* concentrations declined beyond 1.5 m from the end row. An exponential decline in pollen presence over distance has previously been noted (Turner and Brown 2004).

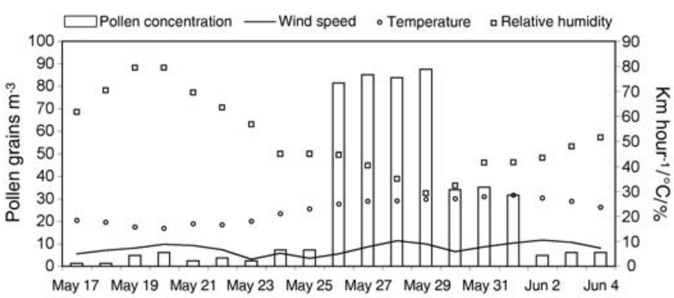

Figure 2 Daily Cabernet Sauvignon pollen concentrations recorded by a fixed sampler installed in the vineyard. Daily mean wind speed, mean temperature, mean relative humidity, and mean wind direction are shown.

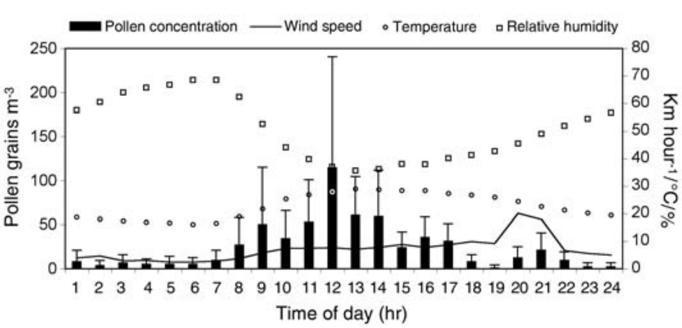

Figure 3 Mean concentration of Cabernet Sauvignon pollen at particular times of the day and 95% confidence interval obtained from May 17 to June 4. Hourly mean wind speed, mean temperature, and mean relative humidity are shown.

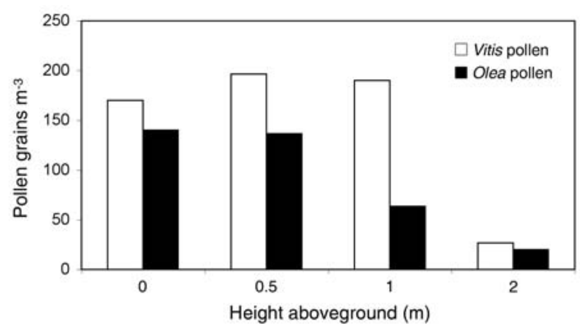

Figure 4 Concentration of Cabernet Sauvignon pollen and olive pollen recorded by portable samplers at particular heights aboveground: 0, 0.5, 1, and 2 m.

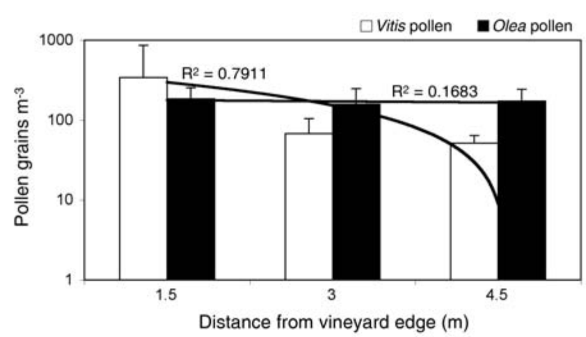

Figure 5 Mean concentration and standard deviation of grapevine and olive pollen recorded by portable samplers at particular points of the Cabernet Sauvignon vineyard: at 1.5 m, 3 m, and 4.5 m from the rows of grapevine. The regression line and R^2 value are shown.

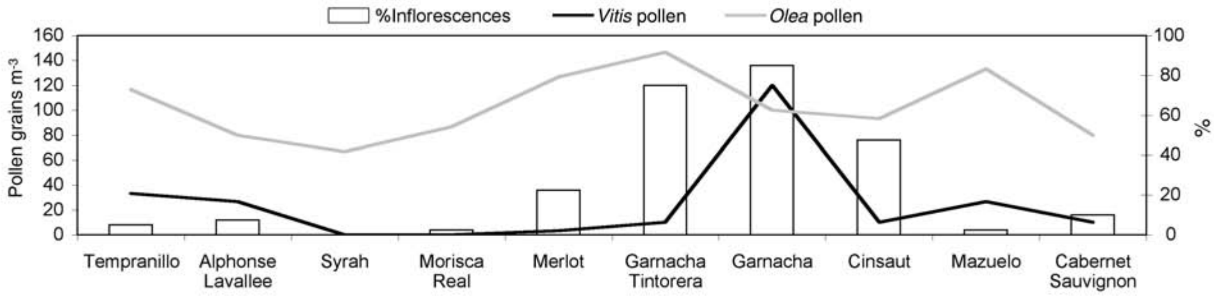

Figure 6 Percentage of inflorescences with open flowers and concentrations of grapevine and olive pollen recorded on 22 May by portable samplers located between the rows of the 10 cultivars.

Pollen concentrations on 22 May were less than 50 grains/m^3 at all points, except in the rows of Garnacha, which had 120 grains/m^3, consistent with its 85% of inflorescences with open flowers, and without a parallel increase in olive pollen, since there was no significant correlation between the concentrations of both pollen types (r = 0.057, p = 0.8852) (Figure 6). Once again, this study emphasized the short horizontal dispersal distance of grapevine pollen, since the pollination peak for Garnacha on this date was not reflected in the concentrations recorded by samplers in the rows of Cinsaut and Garnacha Tintorera, located 4.5 m and 7.5 m from the closest rows of Garnacha, respectively. It is curious that the three other cultivars whose percentage of inflorescences with open flowers exceeded 10% by 22 May had still not reached peak pollination, which could be explained by the fact that the complete opening of the flowers of an inflorescence occurs six days after the start of flower opening (Staudt 1999).

These results assist in interpreting findings that the quantitative and qualitative yield of the Picolit cultivar of *Vitis vinifera*, which is functionally female, was directly correlated to the distance from the pollinator Rebula cultivar (Rusjan et al. 2001). Also, in open areas the large majority of seeds of the dioecious *Vitis vinifera* subsp. *sylvestris* were pollinated by the nearest male located a few meters away (Di Vecchi-Staraz et al. 2009). In the same way, the rate of outcrossing, determined by analyzing seedlings originated from open pollinated vines, reached ~2% at a distance of 10 m and 2.7% at 20 m from the transgenic Dornfelder pollen donor plants in the prevailing wind (Harst et al. 2009a, 2009b).

However, it seems that this finding cannot be applied to other grapevine species, such as functionally dioecious *V. coignetiae*, in which no correlation was found between the rate of maturation and the distance to the male plants when measured at 1 m, 2 m, 4 m, and 8 m, and taking into account four transects in four directions (Kimura et al. 1997). A later work concluded that anemophily was less a factor in pollination than entomophily in this species (Kimura et al. 1998).

Overall, the above results indicate that the dispersal capability of grapevine pollen is low, which contrasts with its characteristics as an anemophilous crop. Taking into account that *Vitis* species still possess features that recall their possible entomophilous origin, such as the production of sterile pollen by female flowers in the dioecious species and the production of scented discharges (Brantjes 1978), the supposition is that it is a group in transition, in which human selection of cultivars with a strong autogamous capability (McGregor 1976, Staudt 1999) has kept the mechanisms of pollen dispersal in an intermediate state. Therefore, the grapevine (*Vitis vinifera*) must be seen as a mainly anemophilous cultivar, since pollination with insects seems to be appropriate only in other species of the genus and its flowers are unattractive to bees (McGregor 1976). But in contrast to other anemophilous cultivars, pollination in this species is only effective at a short distance from the pollen source, so if pollination between compatible cultivars is to take place, they must be planted in close proximity.

Conclusions

The pollination period of Cabernet Sauvignon was seven days, the maximum daily concentration registered between rows in the vineyard at full flowering was 87.5 grains/m^3, and pollen dispersal took place mainly at midday. Daily and hourly concentrations were significant and positively affected by temperature and negatively by relative humidity. Pollen concentration falls drastically between 1 m and 2 m aboveground, and horizontal concentration of pollen peaks at a distance of 1.5 m from the vineyard edge, after which it decreases.

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