Pollen Dispersal Capacity and Pollen Viability of Abies pinsapo Boiss.

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Summary

Fresh- and stored-pollen viability, duration of pollen shedding, and pollen dispersal capacity were studied in a natural *Abies pinsapo* forest located in Sierra de Grazalema (southern Spain). Pollen viability in the fertilization period was high (near 100 %) but was lost after 6 months storage from shedding. Pollen liberation and seed cone receptivity were synchronized within a tree. Total duration of pollen liberation in the forest was 1 month, and in a determinate tree was 6 days. Wind-borne pollen density was studied by means of pollen-traps in 2 neighbouring pinsapo populations with markedly different stand densities. Air pollen density in open forest was remarkably lower than in closed forest. Nearness of some pollen-traps (placed in open forest) to closed forest, and the lower pollen quantities recorded in them, could denote a short-distance pollen dispersal. The results are discussed in relation to the high proportion of empty seeds in some pinsapo populations.

Key words: *Abies pinsapo*, pollen viability, pollen dispersal capacity, plant density, pollen-traps, Andalucía, southern Spain.

FDC: 181.521; 174.7 *Abies pinsapo*; (460); (234.1).

Introduction

Abies pinsapo Boiss. is an endemic conifer of the Sub-Betic mountains of southern Spain (see Fig. 1) that belongs to the group of circum-Mediterranean firs. Pinsapo has a limited distribution (about 1200 ha; Do Amaral Franco, 1950), and is considered a narrow relictic species which is protected in Natural Parks. In some Parks, reforestation programs have been aimed at increasing the distribution area of *Abies pinsapo*, with scarce success up to now.

Pinsapo has a low level of viable seed production in some zones (basically in open pinsapo forest; Arista, 1993). In other conifers, several factors have been suggested or reported to explain low viable seed production: an unduly short effective period of receptivity of seed cones (Villar et al., 1984), early embryo degeneration as a consequence of self-pollination (Owens et al., 1991; Sorensen, 1982), and lack of pollination and consequent lack of fertilization (Owens and Molder, 1977, 1979; Owens et al., 1990, 1991; Colangeli and Owens, 1990). Lack of fertilization (as a result of pollen inviability and/or deficiency of available pollen) seems to be one of the most common causes of empty seed production (Owens and Molder, 1977, 1979; Allison, 1990; Owens et al., 1990, 1991; Colangeli and Owens, 1990).

The purpose of this study was to know the occurrence of some of these processes in a natural forest of *Abies pinsapo* Boiss. Such knowledge is essential for seed production control.

Study Site

The study was conducted in the Nature Reserve of Sierra de Grazalema (36° 45' to 36° 47'N and 5° 28' to 5° 22'W), Cádiz province, southern Spain. Sierra de Grazalema is the area where pinsapo forest is best developed and conserved. There, *Abies pinsapo* forms a dense forest (about 3400 trees/ha) covering 700 ha on the N side of Sierra del Pinar, at an elevation from 900 m to 1400 m (*Fig. 1*). On the higher parts, pinsapo forms pure forest but on the lower forms a mixed woodland with *Quercus rotundifolia* Lam. and *Quercus faginea* Lam. In other parts of the Nature Reserve, pinsapo forms an open forest with a density of about 150 trees per ha. It occurs with *Ceratonia siliqua* L. and *Quercus faginea* and with a dense shrub layer composed mainly of *Leguminoseae, Anacardiaceae* and *Oleaceae*.

The climate of Sierra de Grazalema is typically Mediterranean with mild winter (mean of coldest moth is 6.8 °C) and warm summer (mean of hottest month is 24.6 °C). The main characteristic of the Grazalema climate is its high rainfall with mean annual precipitation about 2000 mm.

Material and Methods

Pollen shedding

Duration of pollen liberation by both male cone and tree was determined by marking 1180 male buds (E- and W-facing) from 5 trees, which were visited twice daily during pollen liberation. On each visit the apertural stage of male cones and the number of opened male cones were recorded. Synchronization of male-female phase was determined by observations of seed cone receptivity in the same trees in the field.

Pollen viability

Fresh pollen grains were collected from pollen cones at the time of pollen shedding from 2 trees about 60 years

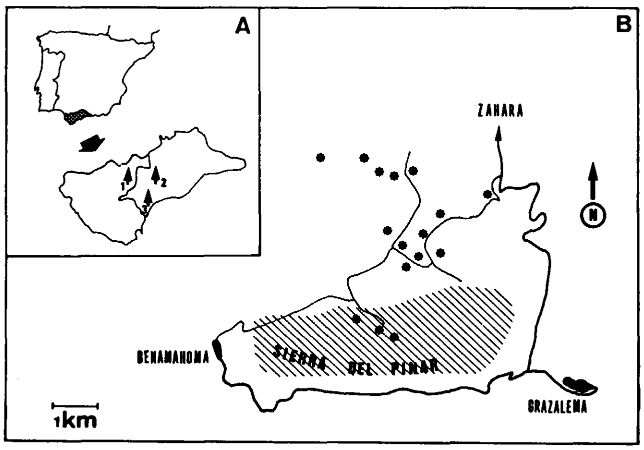

Figure 1. — A) Location of pinsapo forest in the world: 1, Sierra de Grazalema (Cádiz province); 2) Sierra de las Nieves (Málaga province) and 3) Sierra Bermeja (Málaga province). B) Map illustrating pollen-trap positions (•) in Sierra de Grazalema. Hatched zone indicates closed forest and blank zone open forest.

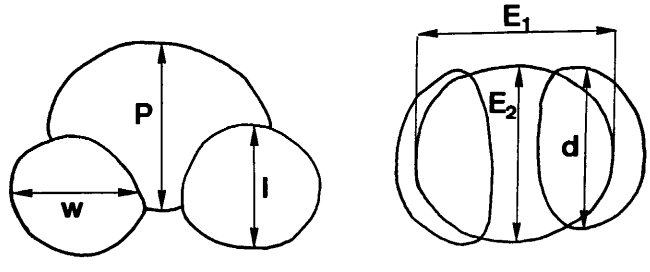

Figure 2. — Equatorial and polar views of the pinsapo pollen grain showing the measured characteristics. P, polar axis; E_1 and E_2 equatorial axes; l, length; w, width and d, depth of the wings.

old. One tree was situated in closed forest and the other in open forest. A portion of the collected pollen grains was mounted in a drop of distilled water and size measurements (*Fig. 2*) were made with a microscope (x 40). The remaining pollen grains were stored at 4 °C in sealed glass vials.

Two months later, at the time of ovule fertilization (ARISTA, 1993), pollen germination was tested in the dark on a solid medium (agar 0.8 %) kept permanently humid by irrigation with distilled water. Experiment was conducted at 26 °C in the dark (because, in vivo, the pollen grains remained ungerminated in the dark enclosed in the seed cones, ARISTA, 1993). Four months later (6 from liberation) a sample of pollen grains stored at 4 °C was sown under the same conditions.

In each sample, 300 pollen grains were visualized at 2-hourly intervals. The number of germinated grains, their morphological changes and the size of the longest pollen tube were recorded. A pollen grain was considered germinated when the size of its pollen tube was half that of the pollen grain body.

Air pollen density

Air pollen density was sampled in 1991 with pollen-traps. These comprised plastic tubes (perimeter of 47 mm), surrounded by a cigarette paper and covered with vaseline. They were placed at a height of 2.5 m on wind-exposed branches. Three pollen-traps were placed in closed forest under the canopy, and 13 in open forest (*Fig. 1*). They were exposed during 5-day intervals from April 15 to June 5, and then collected and transported to the laboratory inside hermetic boxes. In the laboratory, the number of pinsapo pollen grains per a determined surface (625 mm^2) was counted directly on the paper with a microscope (x 40). Pollen grains from other taxa were also identified with the aid of a pollen key (VALDES et al., 1987).

To know daily and altitudinal variation of pollen densities, during May 7, 8 and 9 (at peak of flowering), 6 pollen-traps were placed in a gap of closed forest: 3 at 2.5 m height and 3 at 5 m. These were recorded at 2-hour intervals, and analysed like the others.

Results

Pollen shedding

Branch position was an important factor in pollen shedding. Due to the situation of the studied trees (exposed to the NE), the branches facing east received more sunlight than those facing west. Most sunny pollen cones opened faster than the others. In 48 h, 86 % (N = 518) of east-facing pollen cones opened, while only 50 % of those facing west did so. This difference was significant ($\chi^2 = 20$, $p < 0.05$). Duration of pollen liberation per male cone ranged from 26 h to 53 h (42.2 ± 0.34 h, mean ± standard error).

In the 5 studied trees, duration of the male phase was 6 days. During the time of pollen shedding in the trees, the seed cones were erect with reflexed bracts and ovuliferous scales (a sign of their receptivity; ARISTA, 1993). Therefore, seed cone receptivity was synchronized with pollen shedding within a tree.

Pinsapo pollen has 2 wings and is large (body: P, $\bar{x} = 90.9 \pm 3.5$ μm; E1, $\bar{x} = 97.3 \pm 1$ μm; E2, $\bar{x} = 92.3 \pm 1.2$ μm, n = 22; Wing: depth $\bar{x} = 63.5 \pm 2.4$ μm; length $\bar{x} = 61.9 \pm 1.5$ μm and width $\bar{x} = 61 \pm 3.5$ μm, n = 22; see *Fig. 2*).

Pollen viability

Pollen viability 2 months after shedding was very near 100 %. There was no difference of pollen viability between the 2 studied trees. Pollen germination followed a sigmoid trend, with a similar speed of germination in both trees (KOLMOGOROV-SMIRNOV test, D = 0.3, p = 0.271;

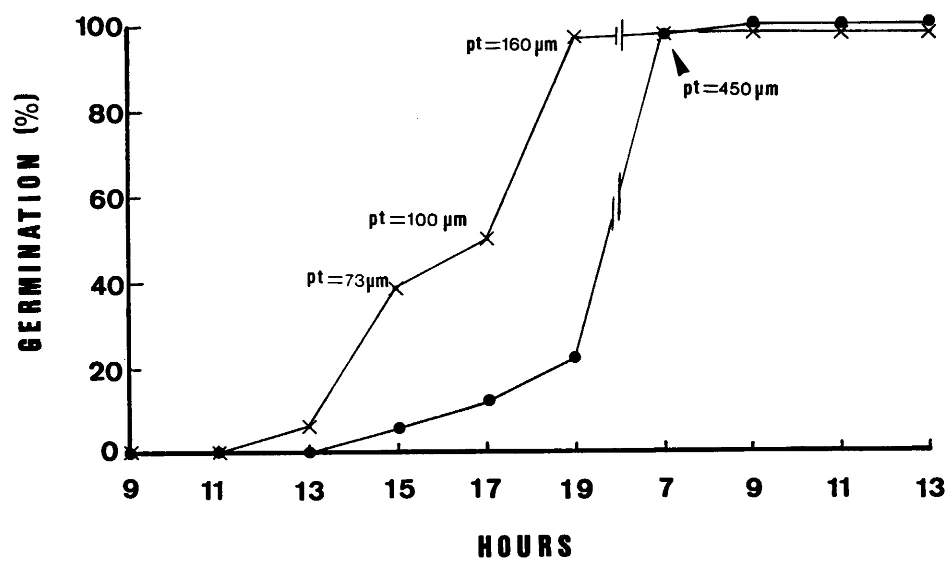

Figure 3. — Variation of percentage of pollen germination with time. In each sampling 300 pollen grains were observed. Maximum size of pollen tube is shown. pt, pollen tube.

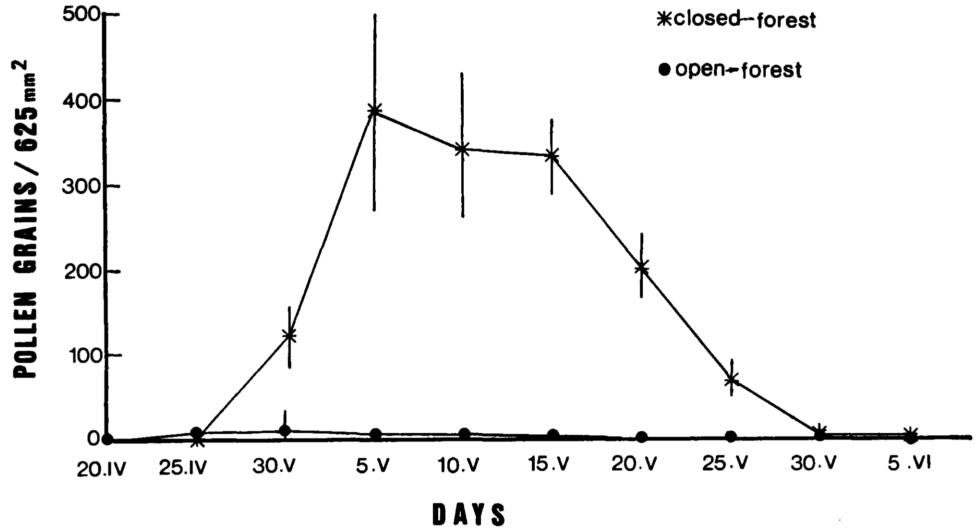

Figure 4. — Air pollen density variation (mean ± standard error) in open and closed pinsapo forest in Sierra de Grazalema during flowering period of 1991. Thirteen pollen-traps were placed in open forest and 3 in closed forest.

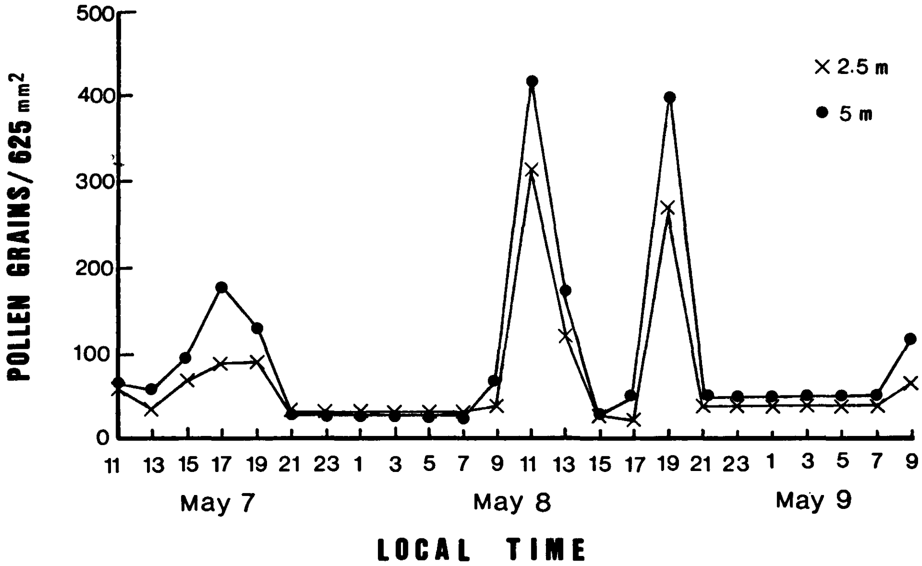

Figure 5. — Daily and altitudinal variation of captured pollen during May 7, 8 and 9 in closed forest of Sierra de Grazalema. Each sample is the mean from 3 pollen-traps.

Fig. 3). Pollen germination slowed for a short period during which pollen grains were hydrated — their volume increased and their nexine disorganized, so the wings were separated and the leptoma shown. Pollen tubes began to burst 10 h after sowing, releasing their content into the medium. At 24 h, between 98 % and 100 % of pollen grains were germinated. The longest pollen tube found was 450 μm long.

Pollen stored for 6 months at 4 °C did not germinate at all. One thousand pollen grains were examined and no pollen tube was found.

Air pollen density

The flowering period of pinsapo in Sierra de Grazalema lasted 1 month. Pollen-traps from open forest began to record pollen grains earlier than traps from closed forest (35 and 0 pollen grains respectively in day 5, *Fig. 4*). Peaks of maximum pollen captured from the 2 zones were separated by 11 days, indicating flowering advance in lower, sunny zones.

Air pollen density of pinsapo in open forest was lower than in closed forest, with a curve hardly rising from the abscissa (*Fig. 4*), indicating a low level of collected pollen. Pollen grains of other species were found in these pollen-traps. Pollen types were: type *Pinus pinea* (represented in the study area by *Pinus pinea* L. and *Pinus pinaster* Aiton); type *Fraxinus angustifolia* (represented by *Phillyrea angustifolia* L. and *P. latifolia* L.); type *Pistacia terebinthus* (represented by *Pistacia terebinthus* L. and *P. lentiscus* L.); type *Quercus coccifera* (represented by *Quercus rotundifolia* Lam.) and type *Quercus suber* (represented by *Quercus faginea* Lam. and *Q. suber* L.). The flowering of these species was synchronized with *Abies pinsapo* in Sierra de Grazalema.

Daily and altitudinal variation of captured pollen in closed forest during 2 days are shown in *figure 5*. Pollen gathered on day 8 was approximately double that on day 7 (1047 and 510 grains respectively). Lower amounts were collected at the end of evening and during the night. On day 7, there was 1 peak of maximum collected pollen between 15 h to 17 h. On day 8, 2 peaks were found: 1 between 9 h to 11 h and the other between 15 h to 17 h. Pollen quantity collected at 5 m height was always greater than that at 2.5 m. Daily trends were similar at 5 m height and 2.5 m (Kolmogorov-Smirnov test, D = 0.2826, p = 0.167).

Discussion

In vitro, pinsapo pollen needs only distilled water and adequate temperature for germination. Similar observations were reported in *Pinus* by Zelles (1979). In other conifers such as *Larix*, in vitro pollen germination is very difficult, and it is necessary to resort to other germination tests (Said et al., 1991). Due to high viability of pinsapo pollen at fertilization time (near 100 %), we conclude that pollen germinability has no effect on the empty seed yield of pinsapo.

Pinsapo pollen lost all its viability after 6 months storage at 4 °C. Possibly, as consequence of the storage method that could be criticized. In most conifers pollen are stored at —20 °C after dehydration (Webber and Bonnet-Masimbert, 1993), and its possible to find better viability rates — for example, 2 years in *Tsuga heterophylla* (Colangeli and Owens, 1991) or 5 years *in Pinus* (Zelles, 1979).

The lower air pollen level found in open pinsapo forest could be an important limiting factor of pollination of isolated trees. A similar situation was found by Owens and Molder (1979) in *Larix occidentalis*. Some pollen-traps placed in open pinsapo forest were very near closed forest (less than 1 km). Pollen grains coming from closed forest could have been captured in the pollen-traps placed in open forest. Thus the pollen-traps nearest to closed forest should have collected more pollen than those placed in open forest. In fact, the amounts collected by open-forest-placed pollen-traps were similar. This suggests a poor suspension of pinsapo pollen in the air, and in consequence a short-distance pollen dispersal. Although the pollen grains have 2 wings, their large size and the high humidity of Sierra Grazalema could be importand factors in reducing suspension. This, and the fact that the pollination mechanism of firs does not seem efficient in collecting large amounts of pollen (Sing and Owens, 1982), could contribute to limiting seed cone pollination in low density populations of *Abies pinsapo*.

Lack of fertilization in conifer forests is usually explained as a result of deficient pollination (e.g., Colangeli and Owens, 1990, 1991; Owens and Molder, 1977, 1979; Owens et al., 1990, 1991; Singh and Owens, 1981). The efficiency of wind pollination is generally assumed to decrease as the concentration of airborne pollen decreases (Whitehead, 1983). This low efficiency may be due to large distances between conspecifics and poor pollen dispersal. In closed pinsapo forest, pollen availability seems to be enough for good seed-set, but high frequencies of empty seeds in low density pinsapo forest (Arista, 1993) could be a direct consequence of lack of pollen. Thus *Abies pinsapo* could have some difficulty in maintaining low density populations. Seed-set in pinsapo populations may be increased using controlled pollination or supplemental pollination (Askew, 1992; Colangeli and Owens, 1990, 1991; Denti and Shoen, 1988; El-Kassaby and Davison, 1990; El-Kassaby et al., 1990; Owens and Molder, 1977; Singh and Owens, 1981). In a determinate tree, controlled pollination ought to be conducted during its pollen shedding, because this is the seed cone receptivity period.

As seed cone receptivity and pollen shedding is synchronized within a tree, self-pollination is very possible (and especially if the pollen do not seem to be transported at a long distance). In some conifers self-pollination is an important cause of empty seeds (Owens et al., 1991; Sorensen, 1982). Due to the nature of this work it is not possible to determine the effect of self-pollination as source of empty seed in *Abies pinsapo*. However, in view of the negative effect of self-pollination in other conifer species, controlled pollination in pinsapo should be conducted with pollen of other trees, increasing genetic gain.

In conclusion, this study confirms a low dispersal capacity of pinsapo pollen, which could be one explanation of the high number of empty seeds in low density pinsapo. forest.

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