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SHORT COMMUNICATION

Pericarp micromorphology and dehydration characteristics of *Quercus suber* L. acorns

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Abstract

The aim of the present study was to examine the micromorphology of the cork-oak (*Quercus suber*) acorn and to evaluate the efficiency of the pericarp as a barrier to water loss. When the desiccation curve obtained at 25 ± 1°C and 62% RH for physiologically mature acorns collected from the tree was compared with that corresponding to fully ripe acorns which had been shed, the latter showed a slightly lower rate of water loss. A marked reduction in the mean rate of water loss was recorded for intact acorns compared with those from which the pericarp had been removed. The external surface and transverse sections of the pericarp from both mature and fully ripe acorns were examined by scanning electron microscopy. Two zones presenting morphological and micromorphological differences were identified: an area including the point of attachment to the cupule and an apical zone covering the embryo. The microstructure of the pericarp showed an external thick cuticle and a single external palisade layer of closely packed cells with no intercellular spaces. Underlying this layer, there was a further parenchymatous layer of poorly differentiated, roughly isodiametric cells. The pericarp in the cupular zone consisted of only this undifferentiated layer between the two epidermal cell layers and contained vascular bundles with many xylem elements.

Keywords: Recalcitrant seed, acorn micromorphology, transpiration, Mediterranean forest, cork-oak, *Quercus suber*

Introduction

The cork-oak *Quercus suber* L. (Fagaceae) may attain a height of 25 m and gives rise to the typical Mediterranean forest as pure stands or mixed with *Quercus ilex* L. and other species of the genus. Growing on sandy soil (Amaral, 1990), its fruits, or acorns (monospermic nuts) contain a large recalcitrant seed (Bonner, 1990; Zulueta and Montoto, 1992; Santos and Bernardino, 1995). In spite of the fact that tree species bearing recalcitrant seeds are generally found in tropical forests, those of the *Quercus* species predominate in the climax phase of the Mediterranean forest. For conservation purposes and even from a strictly economic perspective, knowledge of the mechanisms of water loss from these seeds is important. The term recalcitrant (Roberts, 1973) is presently generally used to describe seeds that lose viability when their moisture content falls to levels between 12% and 31% (wet mass basis). The terms desiccation-sensitive and non-desiccation-sensitive were later proposed by Hanson (1984) to describe recalcitrant and orthodox seeds, respectively. The latter may be desiccated to low moisture contents without significant loss in viability and are thus able to tolerate freezing temperatures.

It is commonly considered that recalcitrant seeds may not tolerate temperatures below zero, but this

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criterion is of an adaptive nature and refers particularly to such seeds of tropical origin. Indeed, recalcitrant seeds of temperate zones are able to tolerate slight frosts, thus restricting the use of this criterion. Therefore, the main factor identifying seeds as recalcitrant is sensitivity to desiccation (Chin, 1995).

Very different methods of preservation are required for recalcitrant and orthodox seeds, with the former, depending on the species, tolerating medium to low temperatures (but above 0°C) and requiring high relative humidity (RH). Seed desiccation is the primary factor to be avoided in maintaining viability. In the specific case of *Quercus* species, preservation of the seed also entails that of the fruit, which is a monospermic nut. The pericarp appears to form the main natural barrier to desiccation under both natural and artificial conditions of seed storage. When the oblong-ellipsoid acorn reaches maturity, it acquires a brown to reddish colour and becomes detached from the cupule, so falling to the ground. Under the prevailing conditions of the south-eastern Iberian peninsula, this occurs from October to December and coincides with one of the characteristic rainfall peaks of the Mediterranean climate.

This investigation was designed to evaluate the efficiency of the pericarp in preventing seed water loss and to examine its microstructure with the following hypotheses in mind:

- the dry pericarp provides a similar or greater degree of protection against desiccation than does the physiologically mature pericarp composed of live cells;
- the pericarp is an efficient barrier to water vapour which reduces the rate of seed desiccation;
- the microstructure of the pericarp is adapted such that it provides a degree of protection against desiccation of the recalcitrant *Q. suber* seed.

Materials and methods

Acorns were collected from a cleared *Quercus suber* wood in the locality of Jaraicejo (Cáceres, Spain). Physiologically mature acorns were harvested from the tree when the pericarp started to change from green to reddish-brown. Further criteria for physiological maturity were the attainment of peak fresh weight and a 94% rate of germination at the time of the study. Fully ripe acorns were obtained from the ground 15 days later. Seeds for the dehydration study were preserved in 30-µm-thick, polythene, hermetically-sealed bags for 24 h prior to analysis.

Acorns for scanning electron microscopy (SEM) were fixed in 2.5% glutaraldehyde for 4 h, washed in phosphate buffer, rinsed in distilled water and placed in a graded acetone series (30, 50, 70, 80, 90, 95, 100%) for 15 min (with two changes). Acorn samples were then mounted onto metal slides and the acetone removed using critical point drying (Balzers, CPD 030), after which they were sputter-coated with gold (Balzers, SCD 004). The external and internal surfaces and transverse sections of the cupular and apical regions of the pericarp were examined and photographed.

Water loss was monitored by maintaining the acorns in a 25 ± 1°C/62% RH chamber in the dark, the original samples having been classified according to size and checked for the absence of fungal infection on the outside of the pericarp using a Zeiss binocular magnifier. Three types of samples were used: (1) intact, physiologically mature acorns (hand-picked from the tree); (2) intact, fully ripe acorns (that had been naturally shed); and (3) fully ripe seeds with the pericarp removed. The acorns and seeds were placed on trays as a single layer and a representative sample taken from each tray every 3 days over a 33 day period. Seed moisture determinations were performed gravimetrically: drying was carried out in an oven at 75°C until constant weight was achieved. Determinations were performed in quadruplicate on batches of six acorns or seeds each time.

Statistical analysis was performed using Statgraphics® software.

Results

Water loss from acorns at different stages of maturity

The physiologically mature acorns collected from the tree had a water content of 49%, significantly greater (P£0.05) than that of those collected from the ground (Fig. 1). Water loss was rapid during the first 72 h in acorns from both samples but greater in those harvested from the tree, until midway through the desiccation process. At this stage, the variation in moisture was significantly different (P£0.05). However, after 21 days of storage at 62% RH, the moisture contents of acorns from the two samples were practically identical. The water loss curves show three stages: the first corresponds to days 1–6 when the moisture content fell rapidly; the second days 6–21, when moisture was lost more slowly, and the third to the stage when water loss was very reduced, presumably because equilibrium with atmospheric RH had been reached.

Efficiency of the pericarp in preventing desiccation

When the rate of desiccation of intact, ripe (fully mature) acorns was compared with that of similar acorns from which the pericarp had been removed,

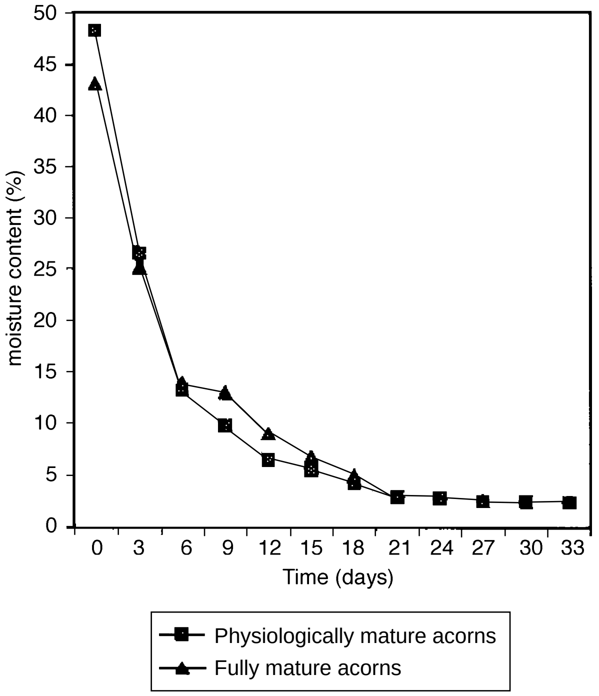

Figure 1. Variation in moisture content of physiologically mature (harvested from the tree) and fully mature (naturally shed) acorns of *Q. suber*, dried at 25°C and 62% RH. Standard errors bars were approximately the same size as the symbols and so are not shown.

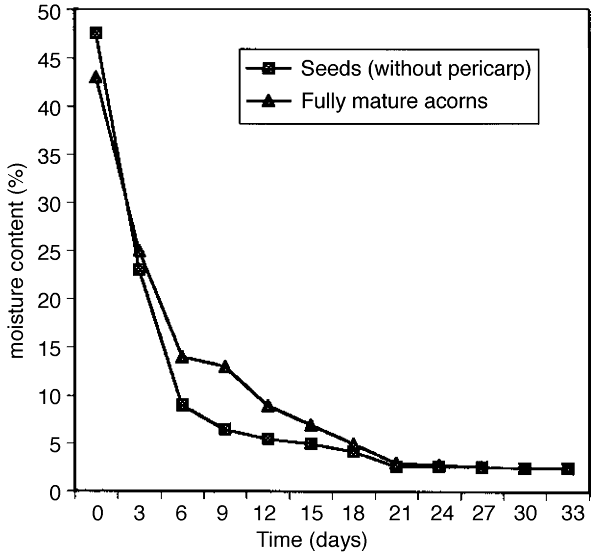

Figure 2. Variation in moisture content of fully mature (naturally shed) acorns and seeds from which the pericarp had been removed, dried at 25°C and 62% RH. Standard errors bars were approximately the same size as the symbols and so are not shown.

significant differences (*P*£0.05) were detected over the first 6 days (Fig. 2). Intact acorns showed a lower rate of water loss than isolated seeds, particularly during the first 6 days of the trial. The corresponding slopes of the desiccation curves became comparable after the seeds had attained a moisture content of 9%, and the acorns of 14%. This pattern continued until the moisture content of both samples became constant, presumably having reached equilibrium with the 62% RH used (Fig. 2).

Morphology and micromormorphology of the acorn

Cupular area

On visual examination, the external surface of the ripe acorn presents two areas of different colour, texture and composition. The cupular surface of the proximal part of the acorn corresponds to the area of attachment to the cupule, which bears bracteoles of axial origin. This structure covers the fruit at the start of development, but encloses it only partially later, a characteristic of *Quercus* spp. The cupule serves to protect the proximal area and is a complex structure. The external surface is covered with bracteoles with long trichomes, while single trichomes covering the internal part are shorter (Fig. 3). The pericarp surface corresponding to the cupule is rough textured, grey and increasingly thickened from the edges to the centre. The vascular bundles enter the acorn at this position and are distributed within an approximately symmetrical, circular crown (Fig. 4). The structure of the acorn cupular area was simple and homogeneous, with the exception of the vascular bundles, being composed of small, undifferentiated parenchymal cells having thick walls but lacking a cuticle or external cell layer, which might afford protection against desiccation.

Vascular bundles show a large number of xylem vessels of different diameter. Some of the larger

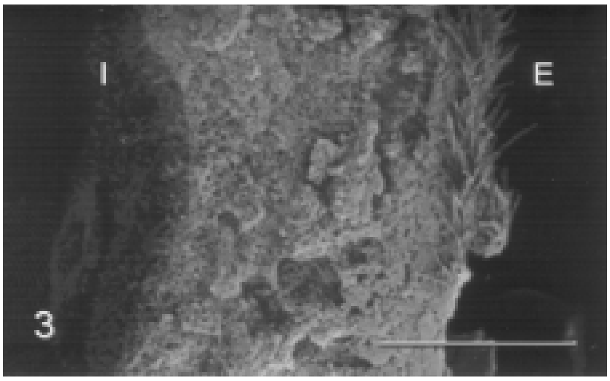

Figure 3. Transverse section through the cupule showing trichomes on the internal (I) and external (E) surfaces. Note the greater length of those on the external surface. Bar = 1 mm.

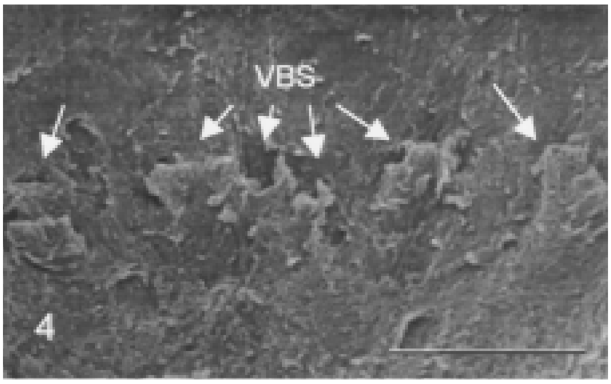

Figure 4. Surface view of the pericarp in the acorn cupular area showing the system of vascular bundles (VBS). Bar = 1 mm.

vessels would have developed during the initial formation of the fruit; but at the time of maturity partial obstructions, interpreted as tyloses, occurred (Fig. 5). Their presence indicates a lack of activity of these vessels when the acorn reaches maturity. Tyloses form by the ingrowth of neighbouring parenchymal cells which penetrate the xylem element (Cortes, 1990). The second xylem element is of the dense, helicoidal type, which occurs in bundles comprised of numerous individuals, and remains active until maturity.

Apical area

The apical area represents the greatest surface of pericarp. It is comprised of a practically smooth cuticle with poorly defined grooves, an absence of pores and dispersed epidermal deposits of wax (Fig. 6). This area changes from green to reddish-brown when the acorn reaches maturity. The cuticle is thick (5 µm mean thickness; Fig. 6) and overlies a thin layer of epidermal cells that are partially embedded in it and hence very difficult to discern (Fig. 6). Underlying the cuticle, there is a dense layer of palisade cells (104 µm average length) with no intercellular spaces (Fig. 7). The layer below is thicker and made up of cells approx. 25 µm ¥ 35 µm in section, with non-thickened walls; the major axis of these cells runs parallel with the surface of the pericarp (Fig. 7). This layer reaches the internal surface and is limited by a layer of interwoven fibrils and not by an inner epidermis. Vascular bundles containing dense, spirally-thickened xylem elements cross the pericarp in the central apical zone of fully mature acorns (Fig. 8).

Apical structure that is essentially similar occurs in the physiologically mature acorns. However, at this stage the cells are alive and completely organised, while in acorns that have been shed, the layer becomes gradually attenuated until palisade cells are no longer evident in the transition band (see below). The parenchymatous layer of the apical area shows a loss of cell organisation after the acorns are

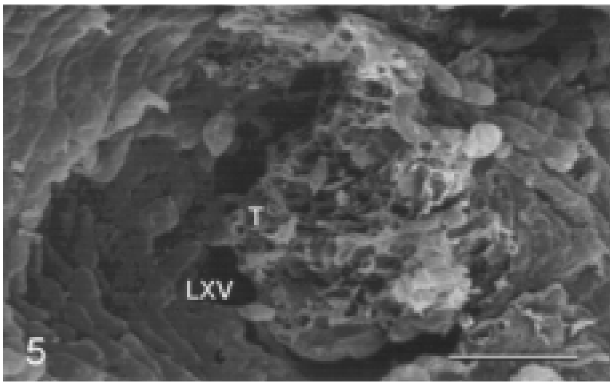

Figure 5. Large xylem vessel (LXV) partially occluded by tyloses (T) in the cupular area of the pericarp of a physiologically mature *Q. suber* acorn. Bar = 50 µm.

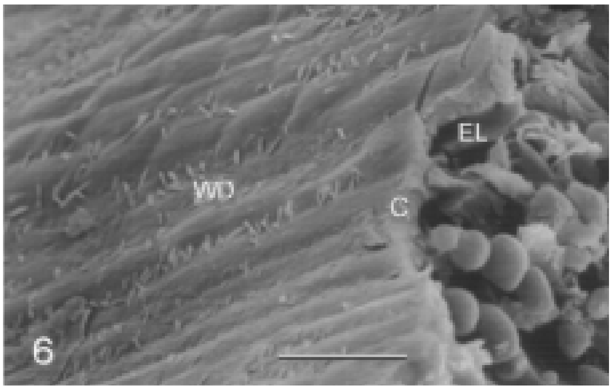

Figure 6. External surface and transverse section of the pericarp of the apical zone of a physiologically mature *Q. suber* acorn. The thick cuticle (C), poorly defined epidermal layer (EL) and strands of wax deposits (WD) are visible. Bar = 20 µm.

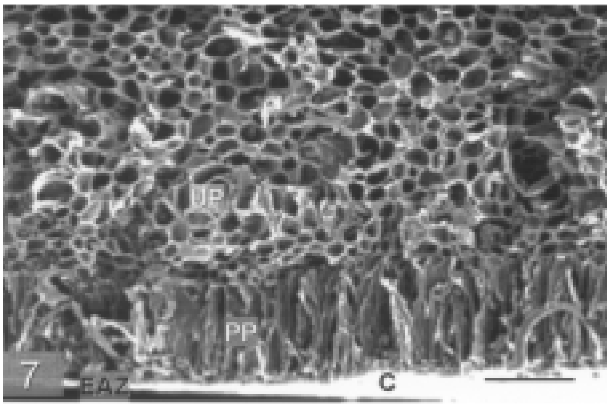

Figure 7. External structure of the pericarp in the apical zone (EAZ) of the physiologically mature acorn. The cuticle (C), palisade parenchyma (PP) and undifferentiated parenchyma (UP) are clearly visible. Bar = 100 µm.

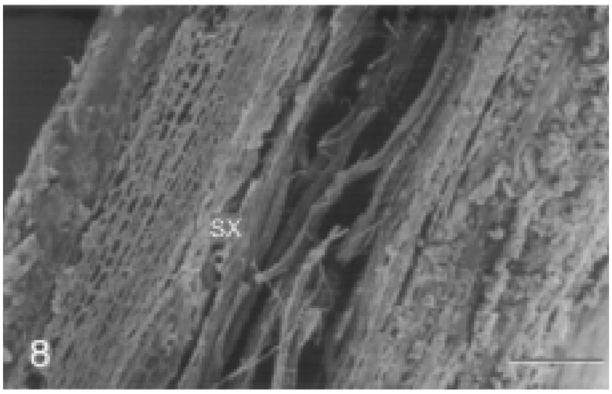

Figure 8. Vascular tissue showing a dense arrangement of spirally thickened xylem elements (SX) in the pericarp in the central apical zone of a fully mature *Q. suber* acorn. Bar = 100 µm.

shed while the cuticle and palisade layers do not.

Transition band

There is a transition band between the two characteristic areas of the acorn pericarp. The apical area slopes towards the cupular zone, which covers it with an overhanging edge (Fig. 9). In the transition band, the cuticle (overlying the epidermal layer) extends to the end of the apical area, while the palisade parenchymal cells become reduced in length some 100 µm from the point of insertion and disappear altogether 10 µm before the junction (Fig. 10).

Discussion

Our findings support the idea that the pericarp constitutes a barrier to water loss, thus affording protection against desiccation to the recalcitrant seed of *Q. suber*. This barrier is functional both during development to physiological maturity and when the fully ripe acorn falls to the ground, thus extending the period of viability of the fruit. After shedding, the parenchymal cells become lignified and die, but the structure of both cuticle and palisade cells is retained. The resultant overall structure of the pericarp of the fully ripe acorns appears to afford a somewhat better barrier against water loss than does the pericarp in the physiologically mature fruit. The palisade parenchyma is interpreted as a barrier to desiccation of the acorn and may have a photosynthetic function during the formation of the fruit. The palisade parenchyma and thick cuticle thus appear to form the two main structural barriers to desiccation. Plant cuticles are described as poorly homogeneous membranes composed of a matrix of polysaccharides and a layer of lipophilic polymers (Holloway, 1994). Diffusion through the cuticle involves the dissolution of the molecules within the lipophilic medium, diffusion in the solid matrix and desorption in the external environment (Lendzian and Kerstiens, 1991).

The moisture loss curves of the three types of specimens are parabolic, with a greater slope shown by the almost perpendicular segment as the rate of moisture loss increases. Comparison of the three curves, however, reveals that in the mature seeds lacking the pericarp the rate of water loss is most rapid, and least rapid in the naturally shed acorns. Thus, although the pericarp varies in its efficiency to slow down water loss in relation to seed developmental status, it does provide a protective barrier, even under the severely dehydrating conditions presently used.

According to our third hypothesis, it was found that the pericarp microstructure is adapted to reducing water loss over most of its surface in the apical area, which constitutes the major surface of the acorn. In this region, the pericarp is mainly comprised of a poorly defined layer of epidermal wax, a thick cuticle which practically envelops the epidermal layer

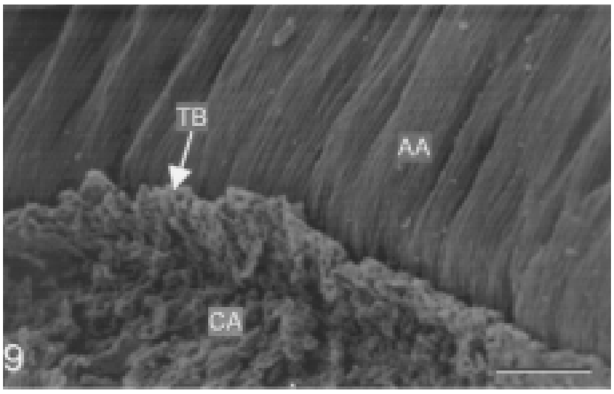

Figure 9. The apical area (AA) sloping towards the cupular zone (CA) of the pericarp is shown. The overhanging edge of the cupular zone (TB) is indicated (arrow). Bar = 100 µm.

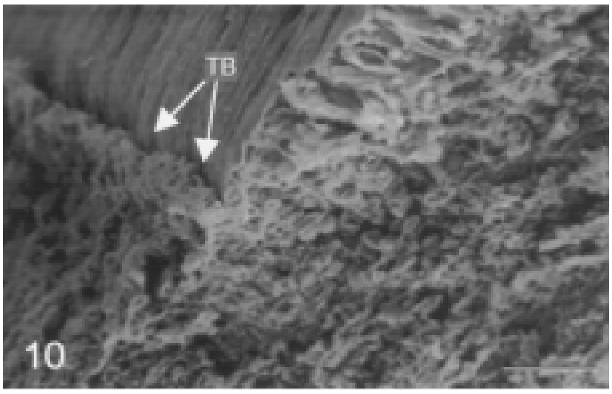

Figure 10. Transverse section of the transition band (TB) of the pericarp. Note the lack of cuticle and attenuating palisade parenchyma in the cupular zone. Bar = 100 µm.

and a single layer of palisade cells. However, the cupular layer, which is traversed by several vascular strands together comprising many xylem elements, lacks this protective structure. Some of the xylem elements are large vessels that presumably facilitate moisture loss, despite their partial occlusion by tyloses, thus interrupting the efficiency of the less permeable tissues of the rest of the pericarp. It is therefore considered that in the cupular area, the loss of moisture is increased since water is presumably able to diffuse continuously from the intercellular spaces to the exterior of the acorn via the xylem elements. In comparison with other non-succulent fruits (e.g. those of *Delphinium*, *Calendula* and *Retama* [Fahn, 1978]), the acorn structure is simple but also relatively efficient in preventing desiccation. The external morphology of the acorn is similar to that of *Castanea sativa*, another dry fruit of the same family (Fagaceae; Rocha, 1990), that is also recalcitrant (Chin and Roberts, 1980).

Protection of the diaspore of *Q. suber* against desiccation is assumed to be at least partially afforded by the particular structure of the pericarp, both during development of the fruit and at full maturity. Thus, it is possible that the overall cupule–acorn system is particularly adapted to preventing water loss when the fruit is on the tree. However, when it falls to the ground the cupule is lost and the fruit is susceptible to increased water loss. Under favourable conditions, cork-oak acorns generally initiate germination immediately after they are shed, which is typical of recalcitrant seeds of a variety of species (Berjak *et al.*, 1989). However, if the autumn–winter temperature is low and the soil is damp, the pericarp should afford efficient protection against water loss. However, when the temperature rises in spring, perhaps accompanied by a decline in soil moisture and atmospheric RH, the pericarp would not be a sufficient barrier to prevent desiccation. Given adequate soil moisture in the spring, the structure of the cupular zone suggests its role in the uptake of water, and thus promoting germination.

From the perspective of the short- to medium-term preservation of this type of recalcitrant seed, the pericarp may be considered a relatively efficient barrier to desiccation within a range of environmental variables. However, under unfavourable conditions, for example those of the present experiments involving the continuously maintained temperature of 25 ± 1°C and 62% RH, the protection afforded by the pericarp against water loss is inadequate. Owing to its structure, the cupular area would seem to constitute the part of the system which is the least efficient at preventing desiccation, but it may have the role of facilitating water absorption during short damp periods or from dew. It would be of interest to determine the difference in permeability of the two different regions of the pericarp and the actual contribution of the microstructure of the cupular zone to the maintenance of viability under natural conditions, with the aim of extending the storage life of cork-oak seeds. Coating of the area of the cupule with waxes or resins might lead to a reduction in water loss, but may also impede gaseous exchange during storage. However, this is a manipulation that is worth testing.

Acknowledgements

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