The aril of *Colophospermum mopane*. Its role during seed germination and fruit opening

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The dispersal unit of *C. mopane* consists of a seed borne within an indehiscent fruit. Both the seed and fruit are short-lived. Under natural environmental conditions the seeds seldom remain viable for more than a year and pericarps of fruits are usually completely degraded after one year. The transitory nature of the testa is reflected in its anatomical structure as it is thin-walled and unlignified at maturity. Seed structure does not conform to that of the typical legume. The thin testa is highly permeable to water and lacks the characteristic malpighian cells and other sclerenchymatous elements. Although water does not penetrate the pericarp of newly-matured fruits, the pericarp soon becomes permeable under natural environmental conditions. Dye experiments using the apoplastic tracer acid fuchsin, revealed that once the fruit becomes permeable, it initially allows water to enter mostly through the ventral and dorsal sutures. With increased scarification of the pericarp, water is able to penetrate over its entire surface. An aril that originates partly from the funicle and partly from the outer integument circumvents the seed. The cells of the aril contain gum-like or mucilaginous substances that swell considerably when they are in contact with water. The aril is situated along the circumference of the seed, in close proximity to the carpel sutures and may aid fruit opening by increasing tension on the fruit valves. The aril is abundantly supplied with vascular bundles as well as with an air canal that forms a highly efficient water transporting system during imbibition. If seeds are removed from fruits prior to imbibition, the aril swells to such an extent that its cells, including the vascular tissue, become disrupted. Imbibitional damage was constantly observed in seedlings that originated from seeds that were removed from fruits prior to imbibition. Seedlings originating from seeds left enclosed in fruits did not show similar damage. In enclosed seeds the aril cells and vascular connections remained intact during imbibition. As the seeds of *C. mopane* are highly water permeable and lack a functional lens to control the rate of water entry, it is proposed that the pericarp governs the passage of water to the seed.

Keywords: seed, anatomy, imbibition, legume, *Colophospermum mopane*.

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Introduction

Colophospermum mopane (Kirk ex Benth.) Kirk ex J. Leonard (mopane), a member of the subfamily Caesalpinioideae, is a xeric leguminous tree species that grows in low (200–800 mm pa) rainfall areas (Mapaure 1994). The dispersal unit of *C. mopane* consists of a one-seeded, oval or reniform indehiscent pod that encloses a flat, convoluted seed (Coates-Palgrave 1983). When the mature fruit is dry it is lightweight and non-woody to allow for easy dispersal. Although the fruit is regarded as indehiscent, it eventually opens along a zone at the ventral sutures of the carpels when moistened. There is, however, no exploding mechanism or twisting of the fruit valves as is usually associated with legumes. Slight bending of the fruit valves, caused by obliquely-arranged fibres of the inner layer of the pericarp, occurs after opening of the fruit and may be indicative of a weak hydrochastic opening mechanism as described by Fahn and Werker (1972) for certain leguminous species.

Permeability of the pericarp is brought about by weathering that allows water to penetrate the pericarp and to be imbibed by the seed tissues. The resultant expansion of the seed inside the locule forces the fruit valves apart and contributes to fruit opening. The fruits open along one side only at a zone of weakness of the ventral suture and not along both sutures as with a typical legume (Roth 1977).

In most cases mature fruits open only far enough along the ventral suture to allow the seed to partially protrude and the radicle to emerge. Fruits usually remain closed while still attached to the mother plant but open fruits and germinating seeds were observed during the onset of the next growing period. Newly mature fruit does not open, despite soaking them in water for

prolonged periods. Not all mature fruits are released from the mother plant. Depending on environmental conditions, delayed seed release may feature in *C. mopane* and is apparently a mechanism to retain a canopy-stored seed bank. Wessels *et al.* (1999) showed the sustained but controlled release of diaspores during different time intervals.

Common attributes related to seed coat dormancy such as structural and chemical impermeability (Kelly *et al.* 1992) does not occur in the seeds of *C. mopane*. In newly formed fruits the pericarp is impermeable to water but, under natural conditions, remains so only briefly. In addition, various anatomical attributes of the seed point to an opportunistic strategy whereby most seeds germinate after a sufficient fall of rain. This strategy involves the rapid and efficient germination of seeds aimed at the establishment of large numbers of seedlings under favourable conditions. Because seeds are already able to germinate soon after their dispersal, they may be referred to as short storage seeds (Gutterman 1993).

There is no information available as to the anatomical attributes or pathway of water uptake of the dispersal unit of *C. mopane*. The aim of this study was to correlate water uptake in seeds that were removed from fruits as well as seeds left enclosed in fruits to certain anatomical features of the seed and fruit that may be connected to imbibition and fruit opening. It is proposed that the aril may fulfill an advantageous role during fruit opening and imbibition, two processes that are crucial for seed germination. In this study some adaptive characteristics of the seed are described in connection with the habitat of *C. mopane*.

Materials and Methods

Seeds of *C. mopane* that were still enclosed in fruits were collected at the Messina Experimental Farm, 20 km west of Messina, South Africa, between 22°12' and 22°17'S and 29°50' and 29°57'. The average annual rainfall for this area is 357 mm. Precipitation occurs mostly in the form of thunder storms and 75% of it occurs between November to March (Decker 1996).

Mature, desiccated seeds cannot be successfully fixed in aqueous fixative due to the cell hygroscopic contents of the aril and seed coat. Intact imbibed and unimbibed seeds were removed from fruits and fixed in a desiccator in osmium tetroxide vapour for 24 h, followed by glutaraldehyde vapour for another 24 h. Fixed seeds were transferred to 70% ethanol for 2 h and then briefly to absolute ethanol prior to being fractured in liquid nitrogen. The fragments were critically point dried, sputter coated with gold and observed with a Hitachi S450 Scanning Electron Microscope at 5 kV.

For light microscopy, almost mature seeds but not fully desiccated were fixed in 4% (w/v) aqueous paraformaldehyde. The material was dehydrated in an ethanol series and embedded in GMA (glycol methacrylate) (O'Brien & McCully 1981). Sections were cut on a Leica RM 2055 Rotary Microtome. Micrographs were taken with a Reichert Univar Photo microscope.

To observe the pathway of water uptake, seeds that were still enclosed in their fruits as well as seeds removed from fruits were placed on sand that was previously wetted with an aqueous solution of acid fuchsin, that acts as an apoplastic tracer. The progressive uptake of the dye was photographed.

To observe the distribution and dimensions of vascular tissue, seeds were cleared in 10% KOH at 100°C for 7 min. They were washed in 3 changes of distilled water, transferred briefly to 70% ethanol and thereafter to 100% lactic acid for approximately 3 hours until the aril was cleared. The tissue was mounted in lactic acid on slides and examined under phase contrast optics with a Reichert Univar Photo microscope to observe the distribution of vascular tissue.

Results and Discussion

The shape of the seed follows the contours of the enclosing fruit wall (Figure 1A). The seeds are reniform in outline and laterally flattened. Before desiccation, a single mature seed fills the locule. The seed may be regarded as ruminate because in the desiccated state the cotyledons are highly convoluted (Figure 1B). The seed coat corresponds to the convolutions of the cotyledons so that the seeds appear highly corrugated in surface view (Figure 1A). The convolutions are greatly reduced in fully imbibed seeds as they undergo a significant volume increase. The raphe and antiraphe are more or less equal in length and the mature seed is campylotropous (Werker 1997). The vascular bundle that is enclosed in a fleshy outgrowth along the raphe-antiraphe does not terminate at the chalaza but circumvents the entire seed. The fleshy outgrowth along the raphe-antiraphe becomes membraneous and wrinkled during seed desiccation. The outgrowth may be referred to as an aril and it functionally partakes in the formation of the testa (Figure 1C). The aril is readily distinguished by its
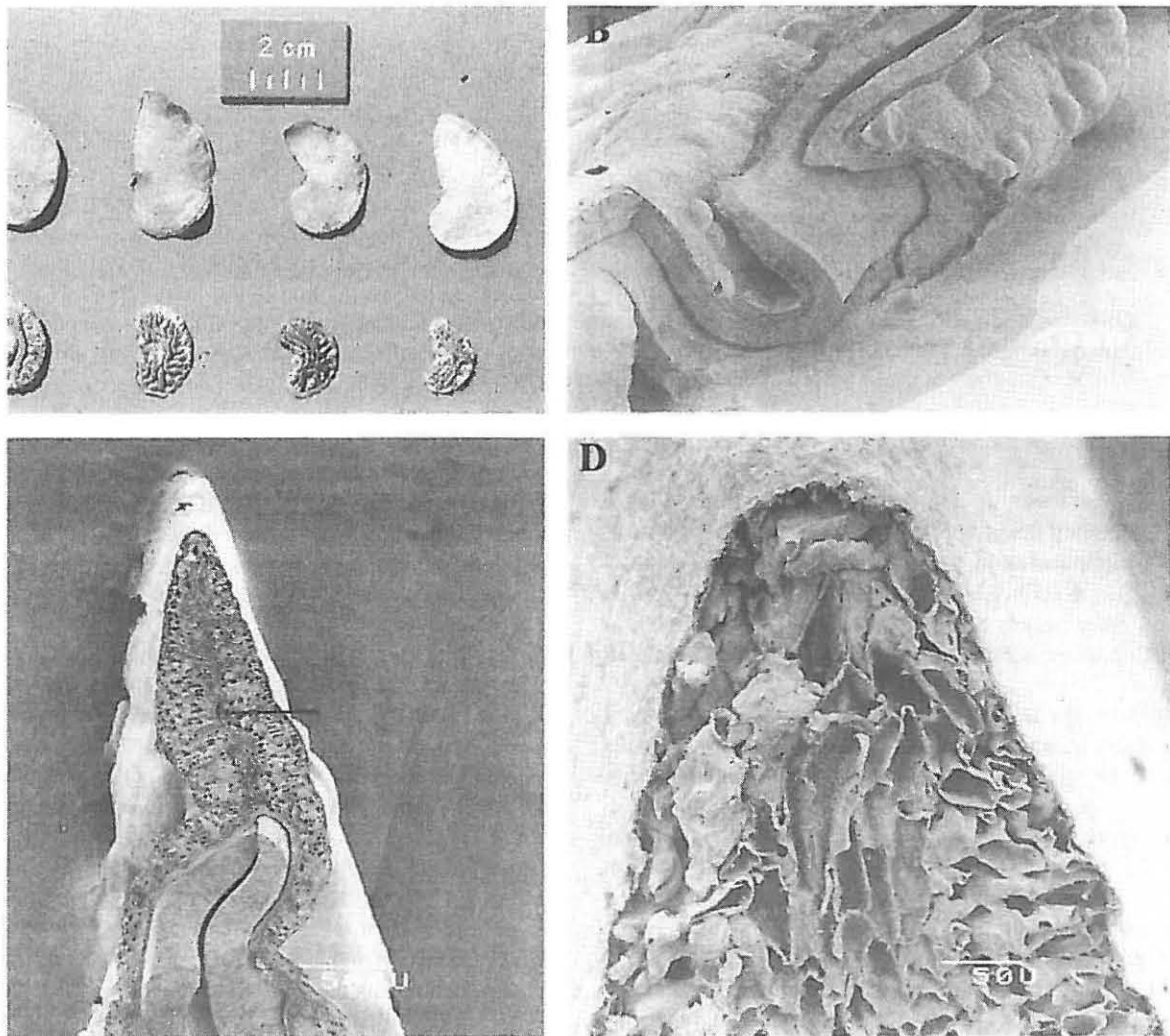

Figure 1 **A.** Fruits with their respective seeds. **B.** SEM micrograph of seed broken in liquid nitrogen to reveal the convoluted cotyledons and thin testa. **C.** SEM micrograph showing a cross section through the aril. The arrow indicates the presence of an air canal. **D.** SEM micrograph of the aril margin to show the thin epidermis and and large sac-like cells.
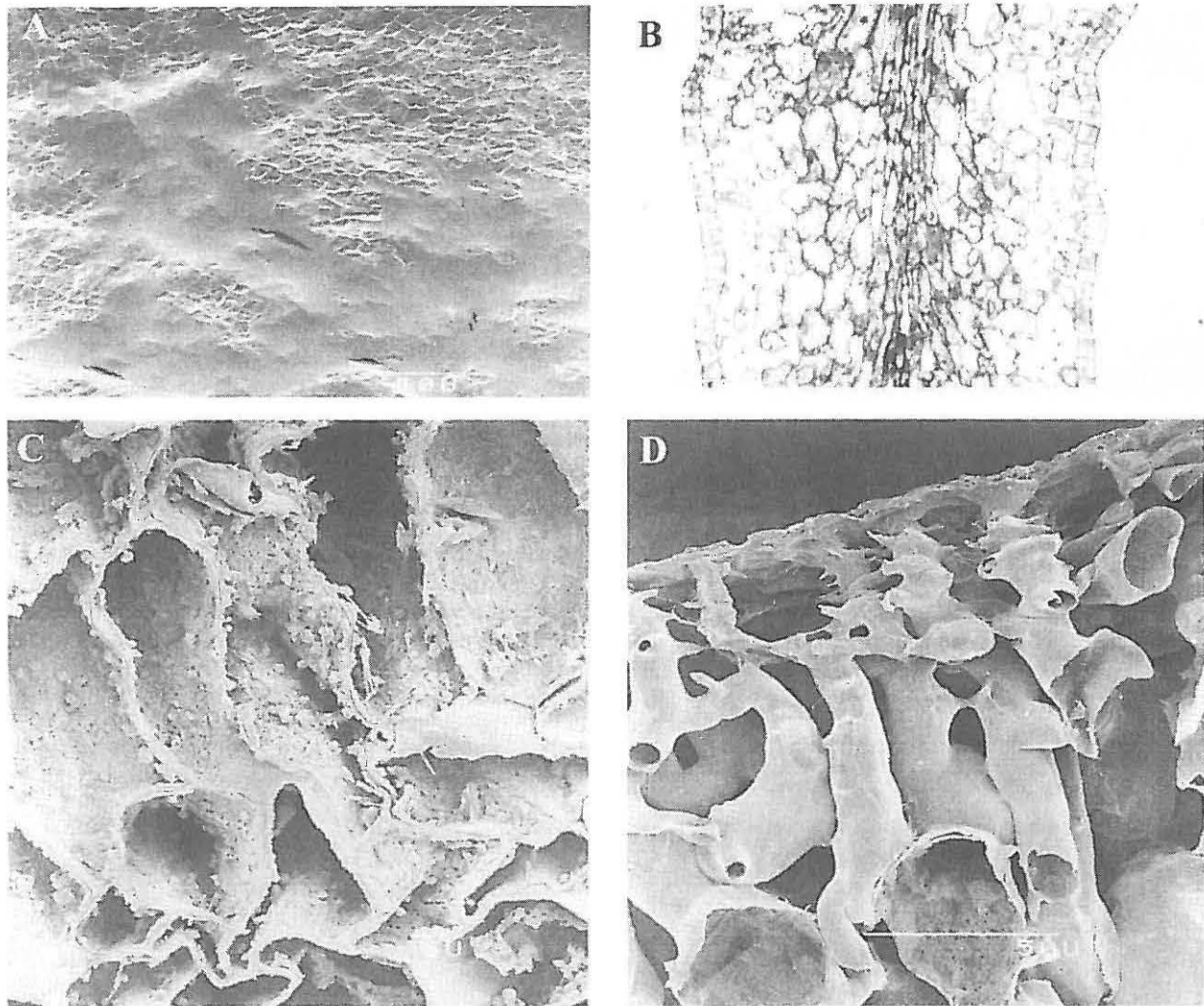
Figure 2 **A**. SEM micrograph of an imbibed seed showing the cuticle that becomes mucilaginous during imbibition of the seed. **B**. Section through aril showing gum-like material in aril cells. **C**. SEM micrograph showing the globular mucilaginous contents of aril cells in unimbibed seed. **D**. SEM micrograph of expanded aril cells after initial stages of imbibition of seeds.

grey colour from the remainder of the thin papery testa which is dark-brown.

Arils have been variously defined (Endress 1973) but Werker (1997) interprets any fleshy appendage of the seed coat as an aril, regardless of its position in the seed. The aril of *C. mopane* is more thoroughly described by Jordaan and Wessels (2000). Arils occur in 14 other genera of the Caesalpinioideae, including in species of *Copaifera*, a close relative of mopane (Van Staden *et al.* 1989)

In *C. mopane*, the seed coat and especially the region constituting the aril is highly permeable to water. Although the aril is fully adnate to the outer integuments, it swells and easily detaches from the seed during imbibition. In addition to the delicate testa (Figure 1D), the substantial permeability to water may be due to the thin cuticle that becomes mucilaginous when wet (Figure 2A). Seed surfaces that become mucilaginous when wet are apparently not uncommon in the legume family. The same phenomenon was observed in *Trogonella oenum-graecum* although the seed did not show a distinct cuticle (Ambegaokar 1976). Hydrophilic mucilaginous- or gum-like material that fills the large cells or sacs of the aril (Figure 2B) may also contribute to water uptake. These large cells develop when the middle lamellae and cell walls, mostly of the centrally situated cells, break down so that their cell contents merge.

Morphologically the aril appears as a fairly inconspicuous structure in the dry state of the seed. SEM micrographs display the mucilaginous content of the aril cells as globules adhering to the cell walls (Figure 2C). In the desiccated state of the seed the cell walls of the parenchymatous aril cells show convolutions (Figure 2C) and the cells are collapsed so that no cell lumen is discernable.

In seeds that have been artificially removed from the fruit and thereafter imbibed, there is a gradual increase in the dimensions of the aril cells so that the cell wall convolutions and wrinkles associated with the dry state disappear (Figure 2D). After one day of imbibition, the large mucilage cells of the aril become globose, separate from one another (Figure 3A) and cause the part of the seed coat cuticle covering the aril to crack or rupture (Figure 3B). Complete disorganization of the different cell layers of the aril is seen with the outer layer and cuticle folding back on itself. In seeds that were imbibed but not removed from fruits, this disruption of the aril only takes place once the fruit has opened. Before this the volume of the aril may increase several times but the cells remain intact and do not detach from the remainder of the testa. When the fruit eventually opens, the aril cells are extruded as a large gelatinous mass from the opened fruit.

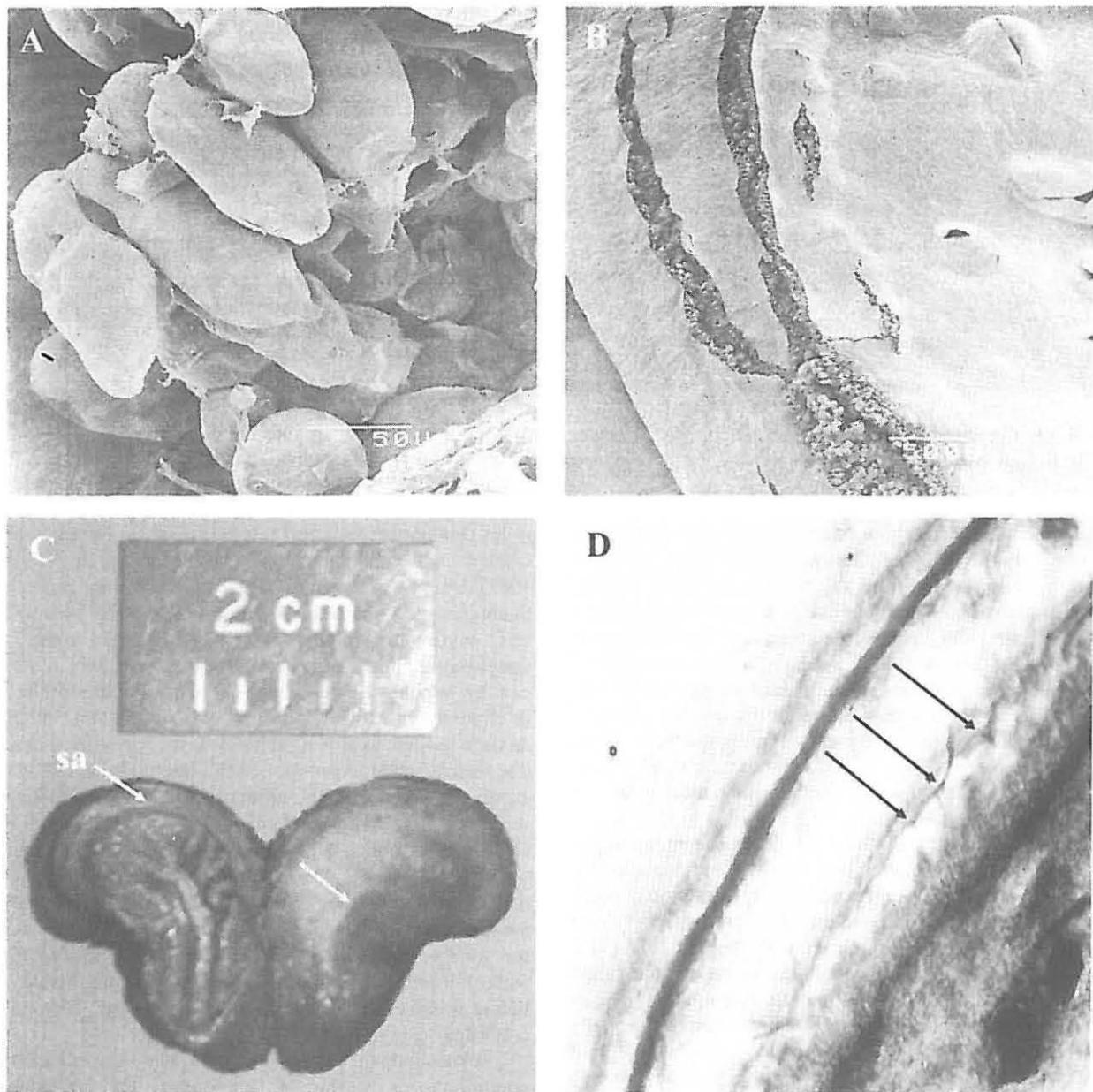Although considered by Boesewinkel and Bouman (1984) to

Figure 3 **A**. Aril cells showing separation from one another and tearing of middle lamellae during advanced stages of imbibition. **B**. Imbibed seed showing cracks in aril after early stages of imbibition. **C**. Areas in fruit where the dye acid fuchsin has penetrated the pericarp (arrow); se. swollen aril. **D**. Cleared seed showing the distribution of vascular connections in aril. Arrows indicate areas where vascular connections were severed from the underlying vascular bundle.

be a rare function of the aril, it seems to be associated in *C. mopane*, with the opening of the fruit. The appendage probably increases tension inside the fruit due to its substantial volume increase and by being situated close to the sutures of the carpels, aids fruit opening. Werker (1997) mentions that mucilage is present in the arils of a number of species but accounts of arils exerting pressure on the fruit valves was mentioned by Boesewinkel and Bouman (1984) to occur only in the Maranthaceae as well as in *Sternbergia clusiana* (Arnaryllidaceae), a desert plant growing in the Negev (Gutterman 1993). Apart from directly exerting pressure on the fruit valves, in *C. mopane* the aril is most likely also indirectly involved in fruit dehiscence. It provides, due to its high hydration capacity and permeability, an expanded surface area that aids seed imbibition and expansion so that the fruit valves are forced apart by the swelling seed.

The volume changes connected to a true imbibition mechanism are based on the ability of cell walls to absorb water, and

cells involved in such a mechanism are usually thick-walled (Fahn & Werker 1972). Since the cells of the aril are relatively thin-walled in mopane, the walls consequently have a small capacity for soaking up water. In *C. mopane* the mucilage cells of the aril differ from typical mucilage cells described elsewhere (Lyshede 1977). True mucilage cells are usually epidermal and the mucilaginous substances constitute secondary cell walls that expand after hydration. Aril cells of *C. mopane* swell mainly due to the mucilaginous contents of the cell lumen and not the cell walls. The volume changes observed in the aril cells when moistened thus resemble a cohesion mechanism (Fahn & Werker 1972). The wrinkling of the cell walls during natural desiccation of the seed is another indication of such a mechanism. The wrinkles typically result as water molecules cohere to one another and adhere to cell walls during natural drying of the seed.

Various ecological functions have been ascribed to seed mucilage, especially with regard to its role during imbibition in dry or
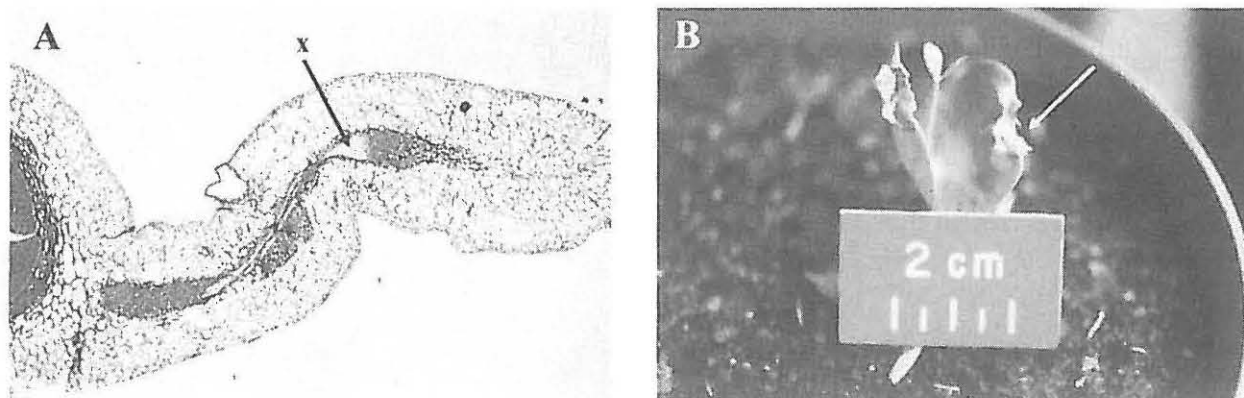
Figure 4   A. Cross section through aril to show the rich vascular supply. The arrow indicates a xylem trace (x) that originates from the funiculus. B. Lesions on cotyledon (arrow) of seedling that originated from seed that was removed from the fruit prior to imbibition.

desert environments, for example, seeds of *Blepharis persica* are capable of germinating at high temperatures when completely covered by hydrated mucilage (Gutterman *et al.* 1973). It is noteworthy to mention that the fruits of certain desert plants belonging to the Brassicaceae that open like *C. mopane* fruits along a special dehiscence zone upon wetting are associated with myxospermous seed (Zohary 1937). Seeds of *C. mopane* cannot strictly be regarded as myxospermous because mucilage is seldom released from the aril and seed coat cells, and the seeds are not glued to the soil as is typical of myxospermous seeds (Werker 1997). Nevertheless, it may point to an active involvement of seed mucilage towards fruit opening mechanisms in plants growing in dry habitats.

Although permeability of the fruit is dependent upon the extent and period of weathering over its entire surface area, the apoplastic tracer acid fuchsin revealed that water mostly penetrated newly formed fruits through the dorsal and especially ventral sutures that are in close contact with the aril cells. This proximity of the mucilaginous aril to the sutures of the carpel may be significant as it allows for the effective imbibition of its cells. The zone of weakness along the ventral suture is especially permeable as is evident from the pronounced uptake of acid fuchsin in this region (Figure 3C). The dye was observed to move through the ventral suture into the placenta and enter the seed through the funiculus from where it entered the vascular tissue of the aril. Water is then delivered to the remainder of the seed coat and to the cotyledons. Older fruits allow water to penetrate not only through the carpel sutures but through the entire surface of the pod from where it is taken up by the seed.

Dye experiments confirmed the role of the aril with regard to the rapid and efficient transport of water along most of the circumference of the seed. The extensive vasculature of the aril, evident from the many ramifications from the main vascular bundle (Figures 3D and 4A), the cambia that continue to form new vascular elements as well as the airfilled canals that act as capillary tubes (Figure 1C), play an important role in this regard. These develop after the vascular supply of some of the vascular bundles are stretched and torn as a result of seed expansion. Jordaan and Wessels (2000) described the vasculature of *C. mopane* seed in more detail. Air-filled canals were also described by Corner (1976) to occur in the vascular bundles of certain members of the Ebenaceae but their origin was attributed to vascular elements that became mucilaginous

In *C. mopane* the extensive vasculature of the aril that is in close proximity with the cotyledons (Figure 3D) as well as the air canals may in effect prove to be a highly efficient conducting system that ensures the rapid imbibition of the seed. During water uptake by the seed, rapid circum-cotyledonary transport of water is important as it prevents uneven stress forces from being exerted on the imbibing cotyledons (Manning & Van Staden 1987). Uneven expansion of the cotyledons may cause imbibitional damage to the embryo. The dye experiment proved the aril of *C. mopane* to be highly efficient in this regard as the dye was seen to enter the aril and circumvent the cotyledons in the matter of a few minutes. However, if the peripheral tissues of the cotyledons are hydrated while the central tissues are not, imbibitional damage, evident as structural injury to the cotyledons, may result due their unequal expansion. Such structural damage was constantly observed in the cotyledons of seedlings produced by seeds that were removed from fruits prior to imbibition (Figure 4B). In such seedlings, the central regions of the cotyledons were not hydrated while the peripheral regions were. During germination the central regions of the cotyledons did not separate from the seed coat. This resulted in the formation of lesions. The reason for this may be that in seeds that were removed from the fruits, the entire aril with its vascular connections were disrupted before water could be delivered to the central regions of the cotyledons (Figure 3C).

It is known that the site and rate of water entry has an effect on seedling vigour (Manning & Van Staden 1987). In legumes, the function of the lens is often related to its role in imbibition and enhancement of seedling vigour as it controls the rate of water entry (Van Staden *et al.* 1989). The lens of *C. mopane* seeds is insignificant and predominantly associated with parenchymatous tissue. As there is no functional lens to control the rate of water entry into the seed, this function may be performed by the pericarp. Thus it seems that once water has penetrated the pericarp and entered the seed, the vascular system of the aril is an important pathway for water transport to the remainder of the seed tissues. In addition to controlling the rate of water entry, the pericarp also provides structural support to the aril so that it remains intact to deliver water to the central regions of the seed. Only after the fruit has opened as a result of expansion of the seed tissues does the aril become disrupted. Once the aril cells are extruded from the pod opening, they may perform the function of preventing dessication of the germinating seed.

Conclusion

The lack of thick-walled sclerenchymatous tissue in the thin-walled testa, as well as the thin pericarp indicates the ephemeral nature of the diaspores. Although thin-walled, the pericarp seems to play an important role in regulating water entry that may otherwise cause imbibitional damage to the seedling. In addition, in

newly formed fruits, it channels water via the ventral suture to the funiculus from where it enters the vascular tissue of the aril. Because the aril is easily disrupted during imbibition, the pericarp provides structural support by keeping it intact until the cotyledons are fully imbibed. The role of the aril in turn, is to provide a transport route for water into the seeds as well as to aid fruit opening by swelling several times its original volume. Total liberation of the seed from the fruit, as was observed in some instances, may have important implications on population number and structure as it reduces the seedlings's competitive advantage.

The tremendous water holding capacity of the aril creates a favourable moisture regime for the germinating seed while still enclosed in the fruit. This may have important implications for the establishment of mopane seedlings in arid environments as the favourable microclimate inside the fruit may enable the seed to germinate under a wide range of available moisture gradients. This conforms to the strategy mentioned earlier that is aimed at high germination efficiency whereby large numbers of seedlings are established when conditions are favourable. The fruit protects the germinating seed even after fruit opening because the opening is only large enough to allow the radicle to emerge and the seed to partially protrude through it. Even then, the disrupted aril cells may still perform the function of protecting the germinating seed from dessication.

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