

**Structure and Water Absorption of Different Parts
of the Rose Achene Pericarp During Imbibition**

S. GUDIN*, LAURENCE ARENE*, A. CHAVAGNAT
and CAMILLE BULLARD*****

* Sélection Meilland, 134 Bd. Francis Meilland, 06600 Antibes – France

** I.N.R.A. Angers, Centre de Recherches Agronomiques d'Angers, Beaucouzé, 49000
Angers – France

*** Laboratoire de Physiologie Végétale, Université de Nice, 28 av. de Valrose, 06034
Nice – France

Abstract. Rose achene imbibition is difficult. Comparison of sulphuric acid treated, Cross and Beavan's reagent (C. B. reagent) treated seeds with untreated seeds showed that, during imbibition, water is essentially found in the most external pericarp layer. Inner layers appear less permeable. It is possible to distinguish three layers in the pericarp by taking into account results from imbibition assays, X-ray radiographies and scanning electron microscopic observations. They can be interpreted as representing the epi-, meso- and endocarp.

Additional index words: *Rosa hybrida* L., Cross and Beavan's reagent, endocarp, epicarp, mesocarp, seed radiographies.

All authors who studied rose achenes germination concluded to an inhibitory effect of the pericarp. However the inhibition characteristics are little understood and except that of Jackson (1968) no interpretation has been proposed. According to Jackson, the inhibiting effect of the pericarp was due to its abscisic acid content. Since then, Svejda and Poapst (1972) and Tillberg (1983) have shown that abscisic acid levels of rose achenes submitted to different post harvest treatments (room or cold temperature stratifications) were similar though their germinability was quite different (no germination after room temperature stratification, significant germination after cold stratification). These results therefore call 1968 Jackson's interpretation in question again.

The aim of the present work was to achieve a better understanding of the pericarp structure and of its water relations during imbibition.

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MATERIALS AND METHODS

Our experimental material was chosen among achenes issued from a crossing *Rosa hybrida* L. cv. Sweet Promise X *R. hybrida* cv. Meinastur carried out in May 1986 at Selection Meilland in Antibes. These achenes were cropped in October 1986 and kept dry 4 °C during 4 months. A screening for density was done by flotation on a hexane – chloroform mixture according to the method described by Taylor *et al.* (1982). Only achenes of density superior to 1 were used. The percent moisture of these achenes was 8.5 %.

Achene imbibition

Five lots of 50 achenes were weighed on a precision scale (Mettler H-80) and placed in Petri dishes on paper filters (Durieux n° 111) hydrated with 10 ml distilled water. The dishes were placed in darkness in a growth chamber continuously maintained at 23 °C and 70 % relative humidity. The achenes mass was determined each 1/2 h during the first 10 h and then each 2 h during the last 14 h of imbibition. Before each weighing, the achenes were roughly dried on a double layer of absorbing paper (Whatman n° 2300916). The imbibition ratio ($\frac{\text{post imbibition mass} - \text{initial mass}}{\text{initial mass}}$) was determined after 20 h.

Sulfuric acid treatment

The achenes were immersed in 95 % sulfuric acid for 1/2 h at room temperature. They were rinsed for 1/2 h under running tap water and cleared by hand of the most external layer of the pericarp. They were then kept on two layers of absorbing paper for 24 h at room temperature.

C. B. reagent treatment

C. B. reagent is made by dissolving zinc chloride (600 g l⁻¹) in 37 % HCl. It was previously used by Lammerts (1946) and Asen and Larsen (1951) to soften achene pericarps of a few rose species in order to isolate embryos and by Ali (1973) and Mac Kelvie and Walker (1975) to scarify rose achenes before stratification and subsequent germination. The achenes were immersed in C. B. reagent for 12 h at laboratory temperature.

X-ray radiographies

The radiography pictures were made on achenes (80 per lot), imbibed or not, previously treated or not with sulfuric acid or C. B. reagent. They were carried out at I.N.R.A. Angers according to the method described by Chavagnat and

Le Lezec (1984) and Chavagnat (1987). The radiography films were used to study the achene structures. Their observation and the measurements were done on a lighted table with a micrometric lens (Haas 8X). Inside each lot, 50 non empty achenes containing fully developed embryos were considered for each kind of measurement.

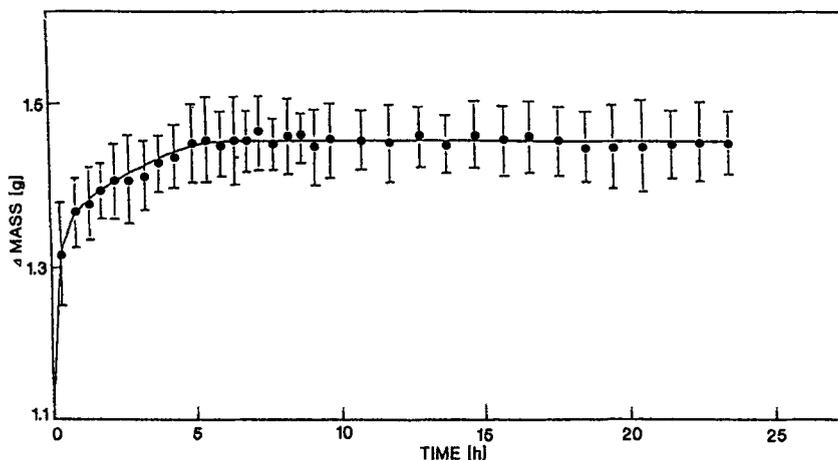


Fig. 1. Fresh mass increase (Δ mass) during imbibition of 250 (5×50) untreated rose achenes.

Scanning electron microscopy

Scanning electron microscopy observations were made at the Service de Microscopie Electronique of C.N.R.S. in Paris VI University by M. Aber. Achenes of density inferior to 1 had been split with a sharp knife and gold metallized before observation.

Table 1

Imbibition ($\frac{\text{Post imbibition mass} - \text{initial mass}}{\text{Initial mass}} \times 100$) after 20 h of untreated achenes and achenes pre-treated with sulfuric acid or C.B. reagent.

	Imbibition [%]
Untreated achenes	16
Sulfuric acid pre-treated achenes	7.6
C.B. reagent pre-treated achenes	40

RESULTS

Achene imbibition

In the case of untreated achenes, increase in water content is low and stabilized after about 5 h (Fig. 1).

We noted that a part of the pericarp is damaged after the sulfuric acid treatment (disappearance of the layer that gives its brown colour to the achene and appearance of a clear subjacent layer). Achenes that had been cleared of the pericarp thin outer layer in this way showed a decreased water uptake compared to that of the reference whole achenes (Table 1). By comparing the absorption rates of the treated and untreated lots, one can see that the outer layer of the pericarp is to a great extent (52.5 %) responsible for the whole achene water uptake.

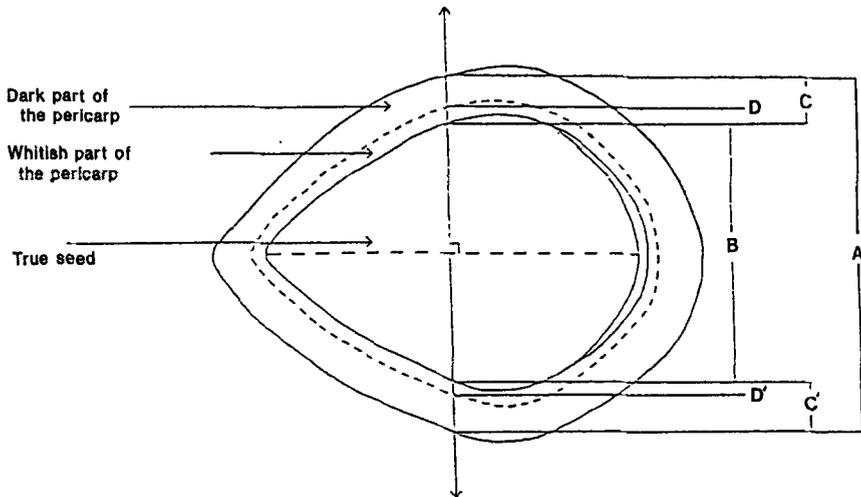


Fig. 2b. Diagram of a rose achene radiography and measurements made on films. A: achene diameter. B: true seed diameter. C + C': pericarp thickness. D + D': pericarp "whitish part" thickness. These measurements are made at the same level by following the true seed longitudinal axis and according to a perpendicular axis which intersects the previous one at its mid point (the term "true seed" applies to the embryo + its seed coat which is not visible on a radiography).

A comparison of the imbibition rates of untreated achenes and C. B. reagent treated achenes (Table 1) clearly shows that the last treatment results in an effective removal of the pericarp impermeability. Since the outer layer of the pericarp is damaged in this case also, a supplementary distructural effect that reaches the inner layer of the pericarp should be also considered.

Study of radiography pictures

The pericarp limits, those of its most internal layer that appears whitish on the radiography film, black on the paper one, (Fig. 2A), and those of the true-seed are clearly visible (Fig. 2B).

Table 2

Measures of achenes structures (mm \pm S.D.) appearing on X-ray radiography films. Means and S.D. result from 50 observations per experimental lot.

Achenes treatments	Achenes diameter	Pericarp thickness	Pericarp "whitish part" thickness
0	4.2 \pm 0.66 (AB)	1.42 \pm 0.4 (A)	0.56 \pm 0.2 (A)
Water imbibition	4.5 \pm 0.66 (B)	1.77 \pm 0.45 (B)	0.54 \pm 0.18 (A)
H ₂ SO ₄ pre-treatment + water imbibition	4 \pm 0.5 (A)	1.24 \pm 0.5 (A)	0.55 \pm 0.19 (A)
C.B. reagent pre-treatment + water imbibition	4.98 \pm 0.66 (C)	2.3 \pm 0.4 (C)	1.08 \pm 0.46 (B)

Means followed by at least one common letter, within a column, do not significantly differ from each other for $P = 0.01$ (Pearson's conformity test).

The diameter of untreated whole achene was not significantly increased after imbibition and was very much smaller than that of achenes previously treated with C. B. reagent (Table 2). Imbibition enhances the thickness of the pericarp in a significant way (comparison of non imbibed and imbibed whole achene). On the other hand no increase is perceptible when the pericarp outer layer has been previously removed (H₂SO₄ pre-treatment). The thickness of the "whitish part" of the pericarp, which corresponds to the most internal layer, only significantly increases in the case of C. B. reagent previously treated achenes.

Scanning electron microscopy results

The three pericarp layers appear very clearly on a scanning microscopy picture (Fig. 3). One can distinguish a specific structure for each layer: the sclerites are tangentially orientated in the most external one while they are radially orientated in the intermediate one; the most internal structure is represented by a thin layer of tangentially orientated sclerites apparently covered with an amorphous pellicle.

DISCUSSION

Imbibition of rose achenes is rapid as Tincker (1935), Semeniuk *et al.* (1963) and Svejda (1972) noted. This observation led Tincker (1935) to conclude that the pericarp does not represent an obstacle to water penetration in this achene. However, the amount of imbibited water in the case of intact whole achenes is very low (16 %), when compared to seat known for other seeds *e.g.* 45 %, 75 % and 90 %, respectively is *Stokesia laevis* (Campbell 1984), *Cyclamen*

persicum (Neveur 1987) and *Citrullus lanatus* (Nerson *et al.* 1985) seeds. In fact, our results show that what can be considered as a "complete imbibition" is only reached when the most internal layer, that corresponds to the least water permeable part of the pericarp, is over-passed.

The different observations we made lead us to propose of a new anatomical definition of the rose achene pericarp. According to the described behavior during water uptake and to the scanning electron microscopy observations, three entities with different characteristics can be distinguished:

- the outer layer of the pericarp, which traps a great amount of the imbibition water and is easily removed after a sulfuric acid treatment, corresponds to the epicarp.
- the internal layer, particularly impermeable to water and appearing "whitish" on radiography films, corresponds to the endocarp.
- the intermediate layer, located between the two previous ones, corresponds to the mesocarp.

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Fig. 2a and 3 at the end the issue.

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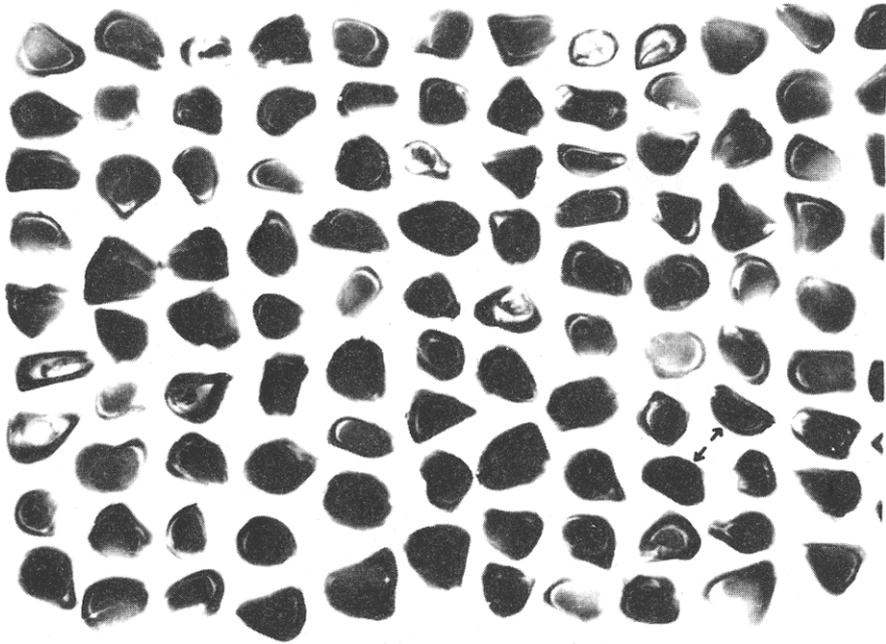


Fig. 2a. Radiography of a rose seed lot. Note the black zone corresponding to the most internal layer of pericarp, different from the rest. It is specially visible on the two achenes pointed with an arrow (this black zone appears "whitish" on the radiography films used for measurements).

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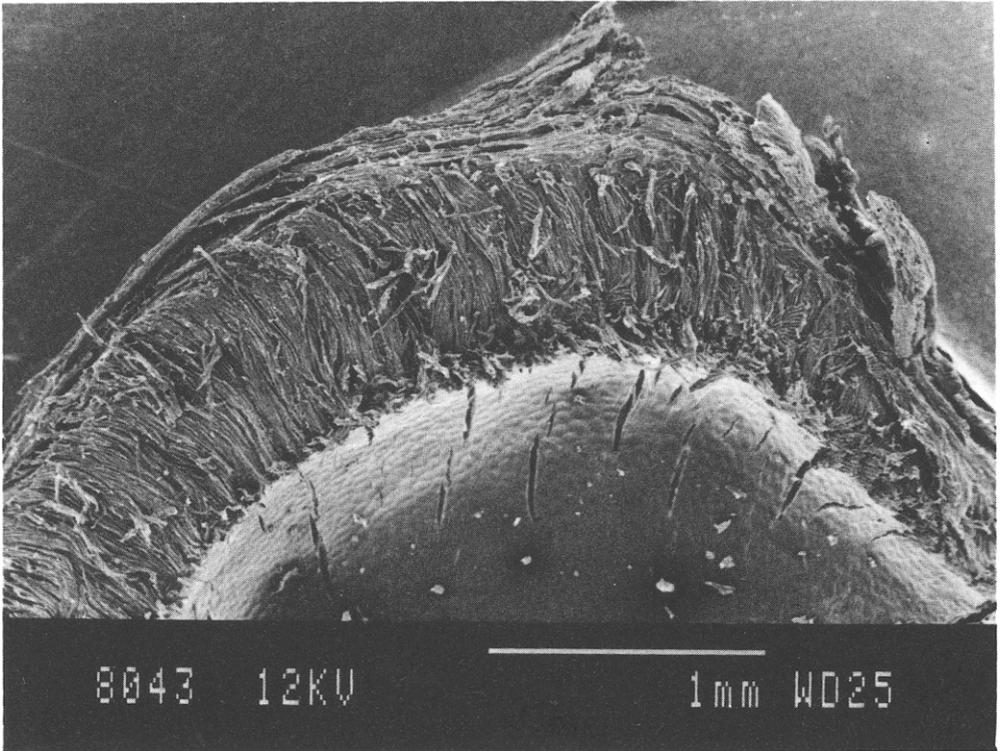


Fig. 3. Scanning electron microscopy picture of the cut surface of an empty achene. Three structures: epi-, meso- and endocarp, can be distinguished inside the pericarp (photo Aber). $\times 33$.