A preliminary study on the germination of *Sclerocarya birrea* subsp. *caffra*

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Seeds of *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro, commonly known as marula, occur in locules within a hard lignified endocarp. The endocarp appears to restrict germination mainly by offering mechanical resistance. It may also restrict the leaching of germination inhibitors and possibly serve as a barrier to oxygen diffusion. The endocarp does not restrict water uptake. High germination counts were only obtained by removing opercula. Leaching slightly improved germination of seeds in intact endocarps. The optimum germination temperature of opercula-removed seeds was between 27° and 37°C. Storage improved rate of germination.
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Sade van *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro, algemeen bekend as maroela, kom voor in vrughokke binne 'n harde gelignifiseerde endokarp. Skynbaar verhoed die endokarp saadkieming hoofsaaklik weens die meganiese weerstand wat dit bied. Dit mag ook die uitloging van kiemingsremstowwe beperk en moontlik weerstand teen die diffusie van suurstof bied. Die endokarp beperk nie wateropname nie. Goeie saadkieming is alleenlik verkry na verwydering van operkulums. Loging het die kieming van sade in intakte endokarpe effens verhoog. Die optimum kiemingstemperatuur van sade, na verwydering van operkulums, was tussen 27° en 37°C. Opberging het die tempo van kieming verhoog.
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Introduction

Some members of the family Anacardiaceae e.g. mango, cashew, pistachio and sumacs are of economic importance. The indigenous marula *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro (Kokwaro & Gillett 1980), a member of the same family, serves as a source of food and shelter for many animal species. It also has ornamental value and plays a distinct role in the cultural rites of various black tribes. They utilize, amongst other things, the seed and especially the fruit extensively. The leaves and bark are used for medicinal purposes (Shone 1979).

Fruit juice and marula liqueur are already commercially produced. Research programmes on cultivation and improvement of marula have been initiated by Prof L.C. Holtzhausen, Department of Horticultural Science, University of Pretoria. As a prerequisite to such studies a thorough knowledge of the germination behaviour of this scientifically little known species is essential.

The fruit of the marula is a highly specialized drupe or stone fruit. The stone is embedded in an edible, fibrous, juicy flesh (mesocarp *sensu lato*) which is covered by the peel (exocarp *sensu lato*). One to four fruit locules occur in the hard lignified endocarp. Each locule contains a single seed, and appears to be a hermetically sealed unit with an orifice tightly closed by a relatively small lid, the operculum. The diameter of the operculum is always smaller than that of the seed and it is therefore completely impossible to remove intact seeds from the fruit. The operculum has to be ejected when germination commences. These opercula have a distinct cream to light brown 'cementing layer', which is more or less weathered in old fruit collected in the veld. The seeds are covered with a thin papery seed coat. The protective function of the seed coat is therefore taken over by the stony endocarp. This endocarp is the effective 'covering' or 'coat' of the marula seed.

In preliminary studies it was found that seeds do not germinate readily. The present study was aimed at identifying the reason for this. Emphasis was placed on the possible restrictive effect of the covering structures. Such structures are known to present chemical and physical barriers to germination (Ballard 1973). In addition the effect of temperature and fruit age on germination was studied.

Materials and Methods

Freshly ripened fallen fruits were picked up beneath trees in the wild at the Nylsvley Nature Reserve (grid reference 2428 DA Nylstroom) during March 1982 and February 1983. The fruit were brought to the laboratory where the peel and flesh were removed. The stones, henceforth termed fruit, were stored at room temperature in screw-capped glass jars. Before use the basal layer of mesocarp adhering to the stony endocarp was removed manually. In some studies opercula were removed by clamping the fruit in a vice and sawing along the 'cementing layer' before lifting them off with a scalpel.

Fruit were germinated in polystyrene dishes (176 × 152 × 35 mm) containing 450 cm^3 washed coarse river sand and covered with aluminium foil. Holes for drainage were pierced into the bottom of the dishes. The sand was moistened with 80 cm^3 of a 0,025% (m/v) Thiulin solution (seed dressing fungicide Bayer S.A.). In preliminary studies this was found to be the most suitable concentration.

Unless otherwise stated, germination tests were conducted in the dark at 27°C and 70% RH. Treatments were replicated four times with 10 fruit per dish, each fruit containing (1) 2 – 3 (4) perfect seeds. The initial germination percentage was calculated on the total number of seeds per dish. To count the total number of seeds per dish in the case of intact fruits, the opercula were removed after the experiment had been completed. The average germination percentage and standard deviation were calculated from the four dishes. Seeds were taken as germinated when the radicle protruded at least 5 mm. Details of other methods are given with the results.

Results

Effect of removal of opercula and leaching on germination

Removing opercula increased germination significantly (Figure 1). Germination of opercula-removed fruits was further increased by a preceding leaching in running tap water for 24 h. Germination of intact fruits was also slightly increased by leaching. However, for significant germination, removal of opercula was a prerequisite (Figure 1). An increase in germination after removing opercula was also obtained when fruits were previously soaked for 24 h in 1 mol dm^{-3} KOH. This treatment, however, did not increase germination of intact fruits (Figure 1). Acid scarification in concentrated sulphuric acid did not increase germination (data not shown).

Water uptake

Water uptake studies were conducted by placing fruits in a paper roll consisting of two sheets of Anchor seed germination paper outside, one layer of Salters absorbant cellulose wadding and one sheet of Anchor seed germination paper inside. Rolls were immersed in distilled water for 10 min whereafter excess water was allowed to drain off. To prevent desiccation, rolls were covered with polyethylene sheeting. Fruits were removed at various intervals, blotted and weighed. After 80 h moisture uptake had levelled off, judged by percentage moisture on an air dry basis (data not shown). Samples of cotyledon tissue were then rapidly removed from all seeds with a cork borer, weighed and then oven-dried at 85°C to constant mass. Removing opercula or damaging the endocarp by rasping did not increase water uptake over an 80 h period (Table 1). By this time some seeds from fruits of which opercula had been removed, showed signs of germination.

Effect of oxygen on germination

Polystyrene dishes containing fruits in Thiulin-moistened sand were placed in glass desiccators. The desiccator lids were modified and provided with gas in- and outlets. Fruits were incubated in air (21% oxygen) and in 100% oxygen. In the latter case 100% oxygen was flushed through the desiccator at a rate of about 2000 cm^3 min^{-1} for 10 min and then sealed. In both cases desiccators were kept sealed for the whole duration of the experiment.

The results in Figure 2 show that raising the initial oxygen concentration to 100% slightly increased germination of intact fruits. Germination of fruits in which the locule had been opened at the base increased significantly. Maximum germination, however, was only obtained with fruits from which opercula had been removed. Raised oxygen concentration did not affect germination of such fruits.

Effect of light on germination

In these experiments intact and opercula-removed fruits were incubated in the light and in the dark at 27°C. The distal parts of the fruits with the opercula were allowed to protrude above the surface of the sand to allow exposure to light. White light was provided by cool white fluorescent tubes (8,25 µE m^{-2} s^{-1}). Only opercula-removed fruits germinated. Germination was better in the dark (72,7% ± 8,7) than in white light (49% ± 14,4).

Effect of temperature on germination

Seed from opercula-removed fruits require high temperatures for germination (Figure 3) with an optimum between 27° and 37°.

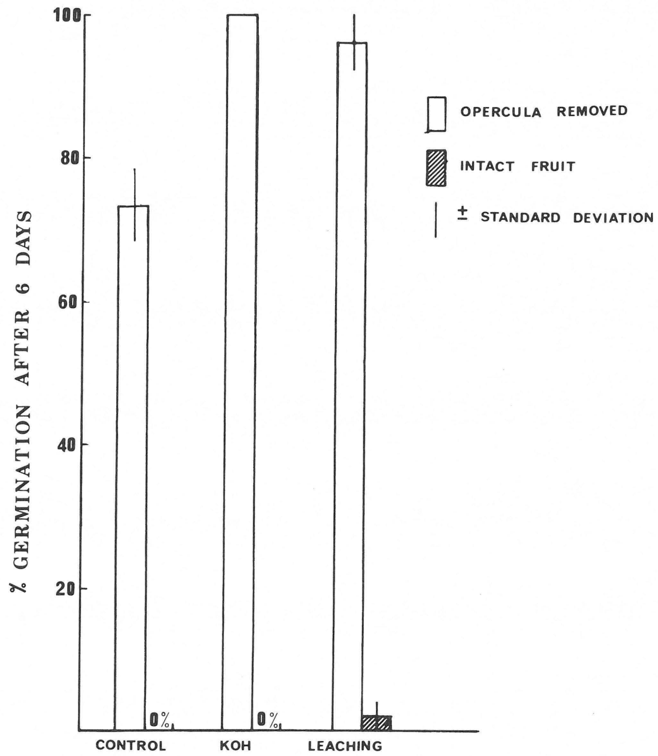

Figure 1 Effect of soaking for 24 h in 1 mol dm^{-3} KOH and tap water leaching (24 h) on germination of marula fruits in the dark at 27°C (fruit age 4,5 months).

Table 1 Moisture content of marula seeds incubated in the dark at 27°C for 80 h (fruit age 6 months)

Fruit treatment	% Moisture content
Intact endocarp	22,5 ± 1,9
Opercula removed	22,4 ± 1,4
Fruit-locule opened at base by rasping	22,8 ± 1,3

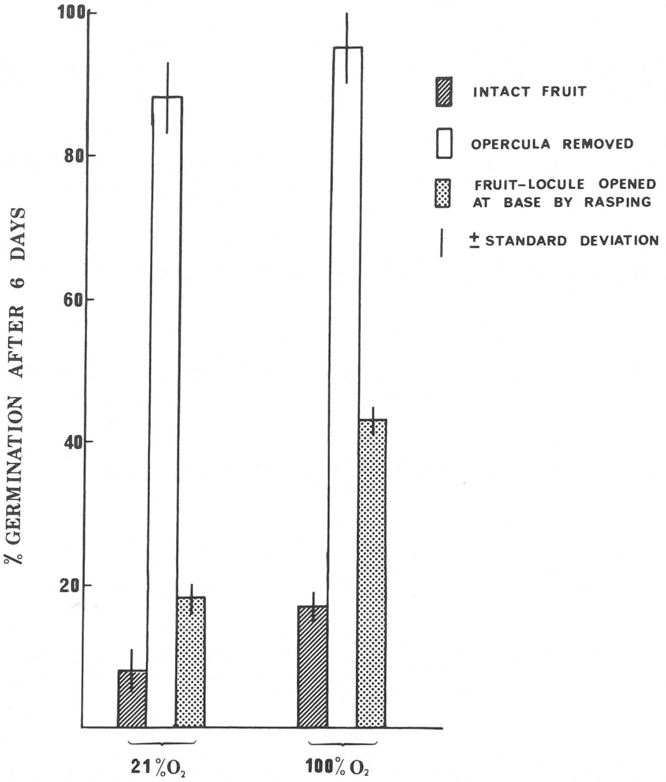

Figure 2 Effect of oxygen on the germination of marula in the dark at 27°C (fruit age 10,5 months).

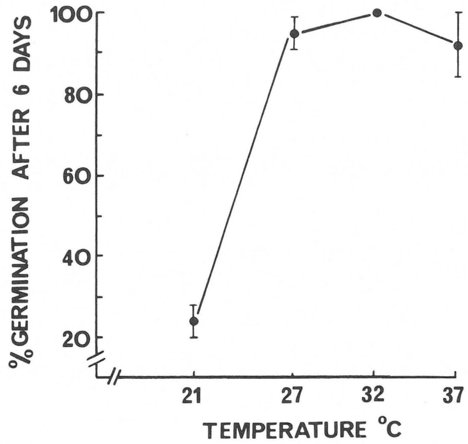

Figure 3 Effect of temperature on germination of opercula-removed fruits in the dark (fruit age 16,25 months).

Effect of fruit age on germination

Fruits were stored in closed glass containers in the laboratory at approximately 21°C. Germination was tested after various storage periods. The results in Table 2 show that with ageing, seeds require progressively shorter incubation times for maximum germination. The fruit lot of 1982 was used for the 14 and 23 months storage periods, while that of 1983 was used for the shorter storage periods.

Discussion

The results obtained in this study show that lack of germination of marula fruits cannot be ascribed to embryo dormancy. In other representatives of the Anacardiaceae e.g. *Rhus trilobata* embryo dormancy occurs and is overcome by cold stratification (Weber *et al.* 1982). The major barrier to germination in marula appears to reside in the mechanical resistance offered by the endocarp. Leaching the fruit with tap water consistently gave a significantly higher germination percentage in comparison with that of the control (Figure 1). It could therefore be reasoned that the endocarp also restricts the leaching of germination inhibiting substances. Another contributing factor of less importance is the barrier offered by the endocarp to oxygen diffusion.

A detailed morphological study of the endocarp of the marula showed that phenolic compounds i.e. tannin-like substances, occur abundantly in the operculum and the remaining part of the endocarp (Von Teichman & Robbertse, in press). Soaking the fruits in water increased the germination rate of *Schinus molle* and *Anacardium occidentale* (Nilsen & Muller 1980; Ibikunle & Komolafe 1973). In *Rhus trilobata* soaking speeded up seedling emergence and significantly increased total seedling emergence (Weber *et al.* 1982). The fact that acid scarification did not increase germination of marula, corresponds to some extent with the results of Nilsen & Muller (1980) on *Schinus terebinthifolius*. However, acid scarification produced significantly higher germination in several *Rhus* species (Morgeneyer 1956; Bear & Sievers 1957; Brinkman 1974; Farmer *et al.* 1982; Weber *et al.* 1982) and *Schinus molle* (Nilsen & Muller 1980). The latter authors stated that the scarification 'might have rendered the bound inhibitors leachable' and suggested an 'endogenous germination inhibition system of phenolic acids' for *Schinus molle* and *S. terebinthifolius*.

The endocarp of marula fruit does not restrict water uptake by seeds. This is in contrast to some other members of the Anacardiaceae (Morgeneyer 1956; Bear & Sievers 1957; Brinkman 1974; Farmer *et al.* 1982).

The slight inhibitory effect of white light on germination observed in this study is interesting and requires further study. In *Rhus glabra* (Anacardiaceae) better germination is obtained in light than in darkness (Brinkman 1974).

The high temperature requirement for germination is

Table 2 Effect of fruit age on germination of opercula-removed fruits in the dark at 27°C

Storage period (months)	% Germination after various incubation periods (days) 3	4	6	12	20
0,5 – 1,0	–	–	23,6 ± 6,4	56,7 ± 2,6	62,7 ± 1,7
2,0	0	5,7 ± 4,0	23,6 ± 8,0	79,7 ± 6,0	91,2 ± 3,0
14,0	17,4 ± 3,4	78,2 ± 12,8	80,3 ± 9,7	*	*
23,0	17,9 ± 11,7	65,4 ± 11,0	80,6 ± 7,0	*	*

– Results not available
* Experiment concluded

consistent with the fact that the marula is a subtropical plant. Unfortunately very little literature seems to be available concerning the optimum germination temperature of other subtropical species of the Anacardiaceae. Rocchetti & Panerai (1968) reported their results on the seed germination of the subtropical *Anacardium occidentale*, i.e. the cashew nut. Stones from Dahomey, Mozambique and Tanzania were germinated in well aerated soil at 95 – 98% RH and at constant temperatures of 10°, 15°, 20°, 25°, 30°, 35° and 40°C. Maximum 'germinability' (i.e. percentage germination) and maximum 'germinative energy' (i.e. expressed in average number of days before germination took place) were reached at 35°C. The moisture content of these *A. occidentale* seeds from Dahomey, Mozambique and Tanzania was 9,93%, 9,57% and 9,67% respectively. The shell, i.e. endocarp of the stones, presented a physical barrier to germination. All these results, therefore, correspond well with the optimum temperature of marula germination, the low moisture content of the marula seed and the mechanical resistance offered by the marula endocarp.

Like some other members of the Anacardiaceae (Brinkman 1974) seeds of marula apparently remain viable for a considerable period. During the present study seeds were found to germinate even after three years of storage in the laboratory.

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