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**Breaking mechanical dormancy in
Quandong using silica gel and enhancing
germination response using Gibberellic acid**

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Abstract

Seeds from the genus *Santalum* are surrounded by a hard and woody fruit endocarp that acts as a physical barrier to radicle emergence. Mechanical dormancy imposed by this endocarp was overcome in *Santalum acuminatum* (Quandong) by soaking and rapidly drying in silica gel, a process proven successful for cracking large quantities of Sandalwood nuts (*Santalum spicatum*). Heating at moderate temperatures was also successful, although a significantly lower percentage of endocarps were cracked. Germination may have been inhibited by a weak embryo dormancy mechanism once the endocarp was cracked, however this was overcome by Gibberellic acid which increased germination to 73 % compared to 42 % in non-gibberellic acid treatments.

Introduction

Mechanical dormancy in seeds is due to the presence of a hard and woody fruit wall, usually the endocarp but sometimes also the mesocarp (Baskin and Baskin 2001). The presence of this hard seed coat mechanically restrains embryo enlargement and/or radicle emergence (Baskin and Baskin 2001). This type of dormancy has been documented in a number of families e.g. Anacardiaceae, Arecaceae, Cornaceae, Elaeagnaceae, Empeteaceae, Juglandaceae, Meliaceae, Nyssaceae, Oleaceae, Rhamnaceae, Rosaceae and Santalaceae (Baskin *et al.* 2002).

Of the 25 *Santalum* species in the world (family Santalaceae), five are endemic to Australia (Woodall 2004). *Santalum acuminatum* or Quandong is one of the most commonly known species, recognised for its 'bush food' qualities in the drier parts of the country. Recent focus has been placed on large scale production not only to harvest its fruit, but also in Western Australia as a valuable contributor to dune and coastal revegetation, an ecosystem where it is often abundant (Tennakoon *et al.* 1997) The endocarp of all *Santalum* species is hard and woody (Woodall 2004). *Santalum acuminatum* has a red fleshy fruit layer (epicarp + mesocarp) that surrounds the hard woody endocarp. Beneath this lies the kernel, a large food reserve (endosperm + embryo) which is covered by a thin woody parchment layer (Figure 1). In this study, the term 'nut' is used to describe the structure that includes the true seed and the surrounding hard and woody endocarp.

Many species with a woody endocarp thought to experience mechanical dormancy, have endocarps that are impermeable to water. This is a physical, rather than mechanical dormancy. In mechanically dormant species, the endocarp is permeable to water but germination does not occur until fruits receive a dormancy-breaking treatment (Baskin and Baskin 2001).

The hard and woody nature of the *Santalum* endocarp limits germination and removing or cracking the endocarp improves germination response. Loveys and Jusaitis (1994) found that the endocarp of *S. acuminatum* acted as a barrier to germination which, when cracked, allowed for radical emergence. Similarly, Woodall (2004) found that cracking the woody endocarp of *S. spicatum* (sandalwood) alleviated embryo restriction. A common production method for cracking the endocarp of *Santalum* species involves applying force with a vice to the micropyle (apex) area, which is the first point of the endocarp to break during germination. The micropyle intersects a depression in the shell which bisects the entire seed along the weakest plain of cleavage (Lethbridge 1995).

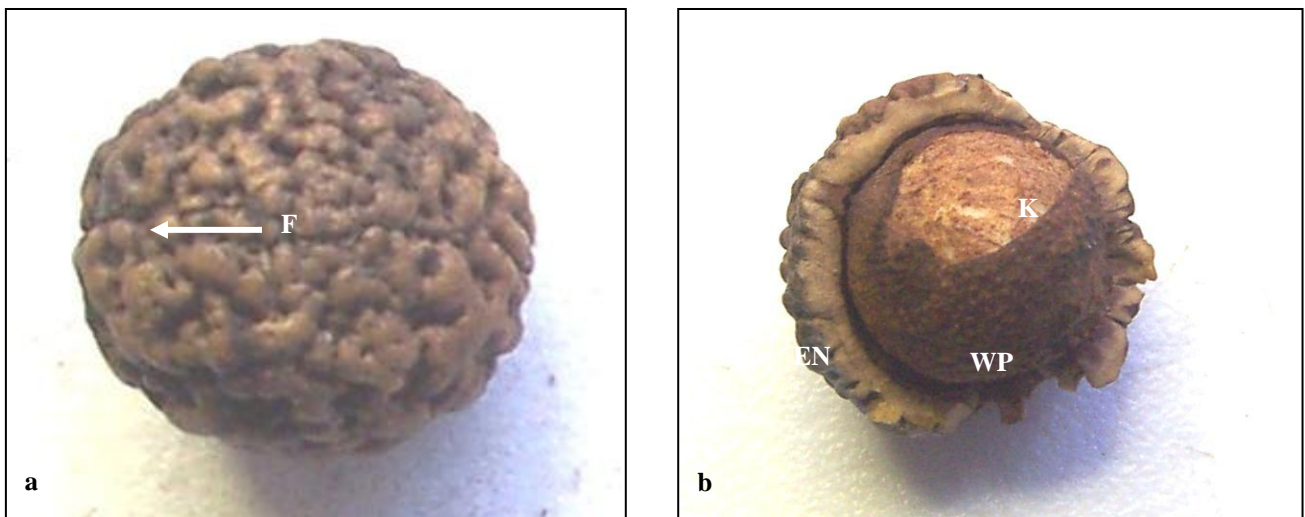


Figure 1. *Santalum acuminatum* (a) Mature fruit showing woody endocarp with small fracture (F). (b) Same nut with endocarp half removed. EN, endocarp; WP, woody parchment; K, kernel (endosperm + embryo).

Others have suggested that nuts be cracked with a hammer, or cut with a saw prior to sowing (Grant and Buttrose 1978). Cracking the endocarp using physical force, although resulting in successful germination, also increases the risk of damage to the kernel. Furthermore, it is a time consuming and inefficient method for cracking large quantities of nuts for production.

The natural mechanisms responsible for removing the barrier of the endocarp were studied by Woodall (2004), who focused on *S. spicatum* nuts. A range of endocarp-cracking treatments were tested in the field and glasshouse including harvest times, wetting durations and drying rates (using heat and silica gel). It was found that rapid changes in nut moisture content fractured the endocarp, allowing for germination. From these results it was concluded that wetting and rapid drying mimics the natural process, where the endocarp is weakened and cracked by cycles of wet summer thunderstorms and hot, dry summer conditions. This wetting and rapid drying technique using silica gel has proved successful in cracking large quantities of *S.spicatum*, *S. acuminatum* and *S. murraynum* nuts (Woodall 2004). Wetting and air drying nuts for seven hours using the draft created in a partially closed fume hood also resulted in cracking of *S.acuminatum* nuts (Loveys and Jusaitis, 1994), however it is unknown what level of cracking success was achieved by this method. It is likely that the use of silica gel is superior to air drying because silica gel drying is quicker and the rate at which drying occurs appears to be the critical factor in endocarp cracking (Woodall, 2004).

Loveys and Jusaitis (1994) suggest that fresh *Santalum acuminatum* nuts in particular, exhibit signs of embryo dormancy (embryo immature at time of dispersal from the mother plant). As a result of this dormancy, Grant and Buttrose (1978) found that germination was better from one-year old seed. Vacuum infiltrating nuts with Gibberellic acid (GA₃ and GA₄) overcame this dormancy successfully, with cracked and GA treated nuts germinating to 90 % after 43 days, compared with approximately 50% germination of cracked and un-treated nuts (Loveys and Jusaitis 1994). If

wetted and dried seeds were subjected to re-wetting, nuts developed secondary dormancy which was also readily overcome by GA.

This paper aims to confirm the most successful cracking procedure for *S.acuminatum*, and investigates the success of Gibberellic acid treatment using a soaking method rather than a vacuum infiltration method for large scale production of *S.acuminatum* seedlings for revegetation in SW Western Australia.

Methods

Seed Source

Santalum acuminatum nuts were collected in late 2004 from Tambellup in SW Western Australia (34 03 S, 117 38 E). Nuts had the flesh removed prior to delivery, and the seedlot consisted of both naturally cracked and uncracked nuts. Upon delivery nuts were stored at 4 °C for approximately one month prior to testing.

Viability Testing

Six samples of 15 nuts were removed for viability cut testing. Three samples of 15 were uncracked nuts and the other three samples were naturally cracked nuts (cracks identified with a magnifying glass (X3). The nuts were then placed in a vice and split into two for examination. Unviable nuts were those which were rotten, discoloured and sticky, desiccated or empty.

Endocarp Cracking Treatments

The following experiments were applied to uncracked nuts. Three samples of 10 nuts were left untreated at room temperature (control). Three samples of 15 nuts were used for each of the four cracking treatments:

- 1) Heated at 40 °C for 24 hours;
- 2) Heated at 55 °C for 48 hours;
- 3) Heated at 70 °C for 48 hours; and
- 4) Rapid drying in Silica gel for 8 hours at room temperature.

Each separate sample (excluding the control) was weighed, then emersed in de-ionised water for 15 hours. Samples were then placed in their respective cracking treatments, and each sample was weighed periodically and the number of cracked nuts recorded until the pre-wetting mass of that sample was reached. The method for silica drying was from Woodall (2004). Samples were completely covered in silica gel in an enclosed container at room temperature until the pre-wetting weight was reached. Only one cycle of wetting and drying was applied.

After reaching their pre-wetting weight, the number of cracked nuts was recorded. Nuts were sown in soil filled trays and placed in a greenhouse with controlled watering for 11 days. It became apparent that seeds were developing a fungal growth so the nuts were re-sown partially buried in coarse sand, with the top quarter of the nut exposed. Trays were placed outside under shade cloth with controlled watering. Nuts with emerging radicles were recorded as germinated on a fortnightly basis between October 2004 and March 2005.

Gibberellic Acid Treatment

Three samples of 15 nuts which had been wetted and cracked using rapid silica gel drying in December 2004, were immersed in 100 mg L⁻¹ GA₃ for 24 hours. Treated nuts were then sown directly into coarse sand, with the top quarter of the nut exposed. Trays were placed outside under shade cloth with controlled watering. The number of germinated nuts (radicle emergence) was recorded in March 2005. Germination was calculated as the number of cracked nuts that germinated as a percentage of the number of cracked nuts sown.

Results

Seed Viability

Seventy percent of uncracked nuts were viable compared with 50 % of naturally cracked nuts. This difference was significant at $p=0.05$ (ANOVA).

Endocarp Cracking Treatments

All treatments were significantly better at cracking uncracked endocarps compared with the control (27 % cracked, Figure 2). Rapid drying using silica gel cracked 73 % of nuts after 7 hours, which was significantly higher than all other treatments ($p<0.001$, Figure 2). Cracking at 70 °C was significantly lower than at both 40 °C and 55 °C. There was no difference in the percentage of cracked nuts between 40 °C and 55 °C.

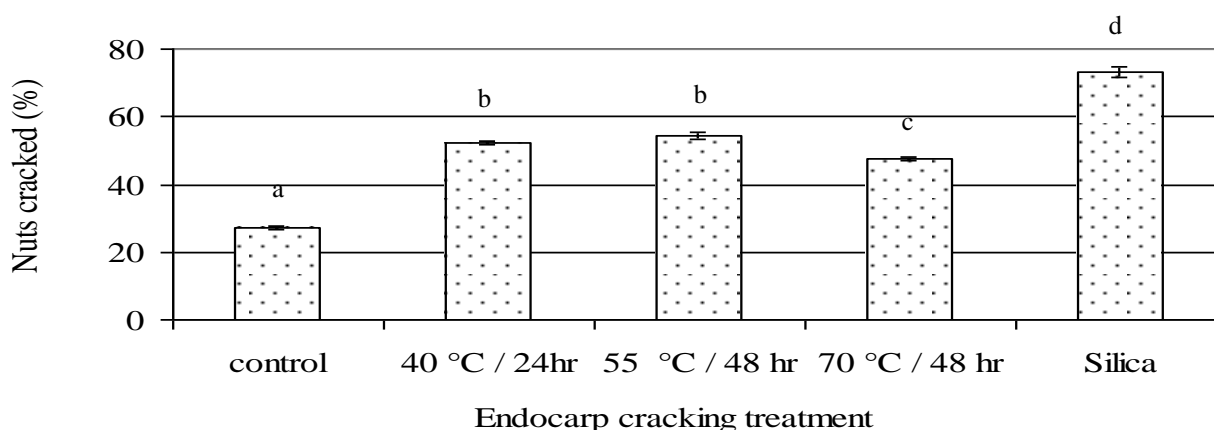


Figure 2. Mean percentage of nuts with cracked endocarp following exposure to 40 °C for 24 hours, 55 °C for 48 hours, 70 °C for 48 hours and rapid drying in silica gel for 8 hours. Bars with the same letter are not significantly different (ANOVA $p<0.05$). Mean bars \pm 1 SE (n=3)

Gibberellic acid treatment

Treatment with GA₃ significantly improved the germination response of cracked nuts from 43.2% in the untreated control, and an average of 46.3 % from other untreated heat and silica drying treatments, to 72.8 % ($p<0.000$, Figure 3). Once nuts were cracked, germination

percentages without GA₃ were the similar (mean 46.3 %) regardless of the cracking method (Figure 3).

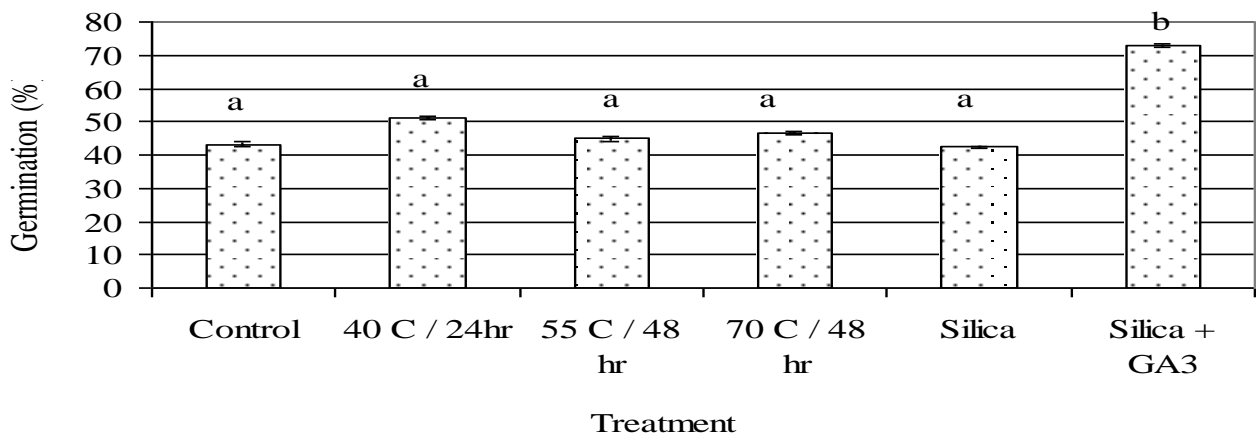


Figure 3 . Mean percentage germination from cracked nuts only, following exposure to 40 °C, 55 °C, 70 °C, rapid drying in silica gel, and rapid drying in silica gel plus GA₃. Bars with the same letter are not significantly different (ANOVA $p < 0.05$). Mean bars ± 1 SE (n=3)

Discussion

One cycle of wetting and rapid drying using silica gel cracked the largest percentage of nuts. Once the nuts were cracked, soaking in 100 mg L⁻¹ of GA₃ for 24 hours significantly increased the germination response from 43.2 % to 72.8 %. The percentage of cracked *S. acuminatum* nuts was lower (73 %) compared with the 98 % cracking of *S. spicatum* nuts achieved by Woodall (2004) with one wetting and drying cycle. Differences between the endocarp of each species may account for this variability (eg thickness). Repetition of the wetting and drying cycle, mimicking natural summer conditions, may result in a higher percentage of cracked nuts. Although this may cause the onset of a secondary dormancy mechanism, this is readily overcome with GA (Loveys and Jusaitis 1994).

Soaking nuts in GA₃, although significantly increasing germination response from untreated nuts, appears less successful than the vacuum infiltration method employed by Loveys and Jusaitis (1994). The latter method resulted in 90 % germination of cracked *S. acuminatum* nuts, 17 % more

than the soaking method in the current experiment. The reason for its success may be due to a greater degree of GA₃ absorption by the true seed. Nuts may also have benefited from germination in a temperature controlled incubator (fluctuation between 5 °C and 15 °C). Nuts in the current experiment were affected by varying degrees of rot and fungal gnats once endocarps were cracked. This was controlled by removing the shade cloth, and application of insecticide (Bayer Confidor 200 SC 0.7ml / L⁻¹). Nut mortality caused by these factors, may have decreased germination.

Gibberellic acid is widely used as a stimulant for germination (Baskin and Baskin, 2001) however its success does not always reflect the presence of embryo dormancy mechanisms. It has been suggested that *S.acuminatum* nuts, particularly fresh ones, are affected by a weak form of morphological dormancy. This is characterised by an under developed embryo at the time of dispersal from the mother plant. This form of dormancy has been documented in many temperate and tropical species belonging to the Santalaceae family (Baskin and Baskin 2001). This form of dormancy can be overcome when treated with GA (Loveys and Jusaitis 1994), as it encourages embryo growth. This growth requirement can result in lengthy incubation periods (the time between sowing and germination). The incubation period in the current experiment was 28 days. Physiological dormancy can also be overcome by GA (Baskin and Baskin 2001). This form of dormancy is caused by inhibiting mechanisms present in the embryo and / or surrounding structures. Warm and cold stratification (Baskin *et al.* 2002), heat pulsing (Tieu and Dixon 2001) and leaching (Copeland and McDonald 2001) may also help in alleviating physiological dormancy. Whether *Santalum acuminatum* suffers from morphological or physiological dormancy has not been confirmed. Leaching has proven ineffective, as has boiling water treatments (Loveys and Jusaitis 1994). Exposure to dry heat (50 °C for 30 minutes) was also ineffective and was found to be inhibitory to germination (Loveys and Jusaitis 1994). This is contrary to the current experiment where treating nuts at 40°C (24hrs), 55 °C and 70 °C (48 hrs) appeared to have no negative effect

on germination. A combination of physiological and morphological dormancy mechanisms may be responsible for low germination. This combination of dormancy can be overcome with GA treatment and warm / cold temperature stratification; further research is required before this can be concluded. Physical dormancy is not a limiting factor for this species. The porous nature of the endocarp and the ability to imbibe water when immersed suggests that lack of oxygen or water is not a significant factor in inhibiting germination (Loveys and Jusaitis 1994).

Interest in large scale production of *S.acuminatum* has grown over recent years. This has led to a need for a reliable and consistently successful method of germination. Wetting and rapidly drying nuts in silica gel has proven to be the most successful method for breaking mechanical dormancy imposed by the hard and woody endocarp in a number of *Santalum* species, including *S.acuminatum*. Subsequent treatment with gibberellic acid, by nut soaking or vacuum infiltration, increases germination. This method may be suitable for other species with a hard and woody endocarp, particularly when species are required in large quantities for fruit production or revegetation purposes.

References

- Baskin C C and Baskin J M 2001 Seeds, ecology, biogeography, and evolution of dormancy and germination. Academic Press: California.
- Baskin C C, Zackrisson O and Baskin J M 2002 Role of warm stratification in promoting germination of seeds of *Empetrum hermaphroditum* (Empetraceae), a circumboreal species with a stony endocarp. American Journal of Botany 89: 486-493
- Copeland L O and McDonald M B 2001 Seed Science and Technology 4th Ed. Kluwer Academic Publishers, London
- Grant W J R and Buttrose M S 1978 Santalum fruit. Domestication of Quandong, *Santalum acuminatum* (R.Br.) A.D.C. Australian Plants 9: 316-318
- Lethbridge B 1995 Current advances in quandong germination. SGAP Journal South Australia 12: 370-371
- Loveys B R and Jusaitis M 1994 Stimulation of germination of Quandong (*Santalum acuminatum*) and other Australian Native Plant Seeds. Australian Journal of Botany 42:565-574
- Tennakoon K U, Pate J S and Arthur D 1997 Ecophysiological Aspects of the Woody Root Hemiparasite *Santalum acuminatum* (R.Br.) A. DC and its Common Hosts in South Western Australia. Annals of Botany 80: 245-256
- Tieu A and Dixon K 2001 A Breakthrough in germinating seeds of Australian Plants. Australian Horticulture, September pp 11-1
- Woodall G 2004 Cracking the woody endocarp of *Santalum spicatum* nuts by wetting and rapid drying improves germination. Australian Journal of Botany 52:163-16