

3 South and Central America

Bolivia (BASFOR, Univ. Gabriel)

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Desiccation and storage of *Anadenanthera colubrina* seeds

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Abstract

IPGRI/DFSC protocol has been used to study *A. colubrina* seeds. The results showed that these seeds could be dried to 4% MC and maintained high viability of 98%. Seeds with >37% MC did not survive longer than 3 months at all tested storage temperatures. However, seeds with <13% MC remained highly viable after storage for 12 months at 18, 4 and -20°C. Their germination capacity decreased during 23 months storage, and only drier seeds with 4 and 8% MC stored at 4 and -20°C maintained about 20% viability.

Introduction

Bolivia is located in the centre of South America with 8 million inhabitants and a surface of 1 098 581 km². Tropical forests cover more than 50% of the country. There are three different geographical areas. The highlands are in altitudes of an average of 3500 m above sea level, with a mean temperature of 5 to 10°C. The valleys are found at 2500 m above sea level, with mean temperature between 15 and 25°C (Pinto 1982). The oriental plains are in altitudes of 350 m above sea level, where the mean temperature is about 30°C. In the highlands, forestry is not well developed because of climatic factors. The most utilized native species are *Buddleja coriacea* and *Polylepis besseri*; exotic *Pinus radiata*, *Cupressus macrocarpa* and *Eucalyptus globules* are also grown. Domestic plans for forestation in the valleys were developed with the help of the international co-operation in the 1970s, '80s and '90s. Several species were planted, but in different proportions, i.e. *Eucalyptus* (47%), *Pinus* (44%) and other species (9%). In the oriental plains, until the last decade, most attention was given to precious species like *Swietenia macrophylla*, *Cedrela odorata* and *Amburana cearensis*. From 1996, the Forest Law reduced their use. In the last five years, forest plantations have increased in these areas, with private initiative and with species of quick growth like *Schizolobium amazonicum*, *S. macrophylla*, *Tectona grandis* and *C. odorata*. However, the exploitation of the forest resources has been indiscriminate, to such extend that species

like *Podocarpus parlatoresi* are greatly endangered and are now listed in the IUCN Red List of Threatened species (IUCN 2002).

The forestry tradition has been to plant well-known species of quick growth, for which there is a guaranteed market for the products. Most of species correspond to exotic species such as *Pinus*, *Eucalyptus* and *T. grandis* and others important species like *S. macrophylla*, *C. odorata* and *A. cearensis*. However, there is a deficiency of knowledge about how to appropriately handle most native forest species. To provide reforestation programs with quality seeds, forest seed banks, such as BASFOR in Cochabamba or the Centro de Investigación en Agricultura Tropical (CIAT) in Santa Cruz, have been established and the new laws have also forced the use of only native species as replacements in natural forests. However, problems were quickly discovered with the germination and storage of forest seeds.

Anadenanthera colubrina (Vell.) Brenan, a native species to Bolivia has been selected to study because of its multiple uses. Its common names are willca, cebil, curupari and curupaú (Killeen *et al.* 1993). This species is dominant and is found in association with *Dodonaea viscosa*, *Schinus molle* and *Myroxylon peruiferum*. The tree reaches 6 m in height and 30 cm in diameter. It is distributed naturally in the central area of the Peru, north of Argentina, Paraguay, northeast of Brazil. In Bolivia it thrives in Cochabamba, Chuquisaca, La Paz, Santa Cruz and Tarija where it can be seen on the hillsides next to the rivers. It grows in secondary forests, on dry and semi-humid inter-andean hills. It grows generally in superficial ground, on drained stony or rocky soil, on steep pending hillsides in altitudes from 315 to 2200 m above sea level. It grows with about 250 to 600 mm per year rainfall with an average temperature of 21°C. The wood has a high calorific value. It is used in construction and for making door and window frames, barrels, mooring masts, hedges, platforms, floors, agricultural implements and railway sleepers. Its by-products are used in medicine and as tannin to harden leathers. It is also known that this species is of quick growth (1–1.5 m per year under favourable conditions). It is recommended as a high-priority species for reforestation programmes.

A. colubrina produces small creamy white flowers, gathered in spherical shapes. The fruits are legumes with flat brown sheaths. The seed is dark brown, flat and circular with two cotyledons covered by a brown testa. Seeds of this species, according to Lorenzi (1992), cannot be stored for more than 4 months, rapidly losing their viability (Torrico *et al.* 1997).

Materials and methods

Fruit collection and processing

The fruits of *A. colubrina* were collected in July 2000 and 2001, from Tín Tín, Mizque, Cochabamba located at 18°22' S latitude and 65°02' W longitude, between 1990 and 2100 m above sea level (Jimenez 1991). Fruits were harvested from 25 trees in a dominant secondary forest and were transported the same day to BASFOR in open polyethylene sacks. The average ambient temperature during transport was 21°C. Seeds were extracted in the shade the second day of collection and immediately dispatched to the replicating partner.

Initial trials

Upon arrival at BASFOR, the initial moisture content was determined and the first samples of seeds were tested for germination. The weights and dimensions of the fruits were determined. Samples of seeds were desiccated to target MCs and also tested for germination (IPGRI/DFSC 1999, 2000). The moisture content was determined by weighing before and after oven drying at 103°C for 17 h, and then calculated as a percentage of fresh mass. Only seeds from the 2000 harvest were treated for 10 min with 5.25% hypochlorite of sodium (20 ml NaOCl for 1 l of water).

Seed weights were monitored until they reached the target moisture contents. Samples were then taken and sown using four replications of 25 seeds in sifted sand, washed and sterilized at 103°C during 17 h. Seeds were sown in rectangular polystyrene boxes with a cover [180×120 mm (base)×70 mm (height)] and put in a germination room at 28–30°C with 8 h light and 60–100% relative humidity.

Results

Initial characteristics

Initial characteristics of *A. colubrina* seeds collected in 2000 and 2001 were similar. Seeds from both lots had initial moisture contents of ca. 41% and germination of ca. 97% (see Table 1).

Table 1. Initial characteristics of *A. colubrina* seeds harvested in 2000 and 2001

| | 2000 seed lot | 2001 seed lot |
|--------------------------|---------------|---------------|
| Fruit size: —length | 19.9±2.6 | 18±3.3 |
| —width (cm±SD) | 2.0±0.2 | 2.1±0.2 |
| Fruit weight (g ± SD) | 7.91±2.2 | 7.23±2.3 |
| Seed weight (g ± SD) | 0.32±0.08 | 0.27±0.7 |
| Seed diameter (cm ± SD) | 1.72±0.19 | 1.66±0.15 |
| MC (%) before processing | 41 | 41.3 |
| MC (%) after processing | 37.0 | 38.2 |
| Initial germination (%) | 97 | 98 |

Desiccation trials

A. colubrina seeds with 36% MC from both the 2000 and 2001 lots, were desiccated down to ca. 7% MC. They all maintained high viability of 98% at this moisture content, showing that these seeds were not desiccation sensitive (Table 2).

Table 2. Germination capacity (G%) of *A. colubrina* seeds after drying to different moisture contents (MC%)

| 2000 seed lot | | 2001 seed lot | |
|---------------|-------|---------------|-------|
| MC (%) | G (%) | MC (%) | G (%) |
| 36.2 | 97 | 35.8 | 97 |
| 32.4 | 97 | 33.2 | 96 |
| 27.5 | 94 | 26.4 | 97 |
| 23.3 | 97 | 22.8 | 97 |
| 13.8 | 95 | 12.9 | 98 |
| 6.9 | 98 | 6.7 | 98 |

Storage trials

The storage results showed that seeds with >37% MC did not survive longer than 3 months at all tested temperatures (Fig. 1). However, seeds with <13% MC remained highly viable after storage for 12 months at 18, 4 and -20°C. In a separate trial, seeds from 2001 lot that were not treated with sodium hypochlorite, had better recovery (76–90%) than the treated seeds of 2000 lot, at all tested temperatures after 11 months storage (Fig. 1). This indicated that NaOCl might have affected the seeds during storage. The germination capacity decreased during storage up to 23 months, and only drier seeds with 4 and 8% MC stored at 4 and -20°C maintained about 20% viability after this time.

Discussion

This study showed that seeds of *A. colubrina* were not desiccation sensitive as previously suggested (Lorenzi 1992). They were dried to 4% MC and maintained high viability of 98%, and were stored for more than a year at 18, 4 and -20°C (Table 2 and Fig. 1). It was suspected in the first year (2000) that sodium hypochlorite damaged and affected viability, maybe due to the high concentration. Thus seeds of the 2001 collection were not treated, and the 11 months storage results were highly improved, 76–90% against *ca.* 50% (Fig. 1). After 23 months storage, only drier seeds with 4 and 8% MC stored at 4 and -20°C maintained about 20% viability.

Additionally, this study allowed collection of data on the phenology, ecology, uses and yields in laboratory and in nursery. These results will help produce seedlings for reforestation and provide another choice of species for planting. There is therefore a potential for *ex situ* conservation of this species, as well as *in situ* conservation. Such a study can be extended to other important species like the *Aspidosperma quebracho blanco* or endangered species like *Podocarpus parlatoresi* (IUCN 2002), for which there is hardly any knowledge of the biology of their seeds. This would raise BASFOR expertise in the handling of forest seeds as a leading institute in Bolivia.

Collaboration within the project

The project of recalcitrant seeds has been well implemented in BASFOR. The training workshop for the protocol held in Costa Rica in 2000 was useful. The workshop gave a good opportunity to meet other scientists from different countries and to build institutional collaborations. The *modus operandi* was very efficient from all the participating institutions within the project and no serious problem was encountered with logistics and finances. The co-ordination with the replicating partner within Bolivia was good because of the training workshop that put together all the contributors.

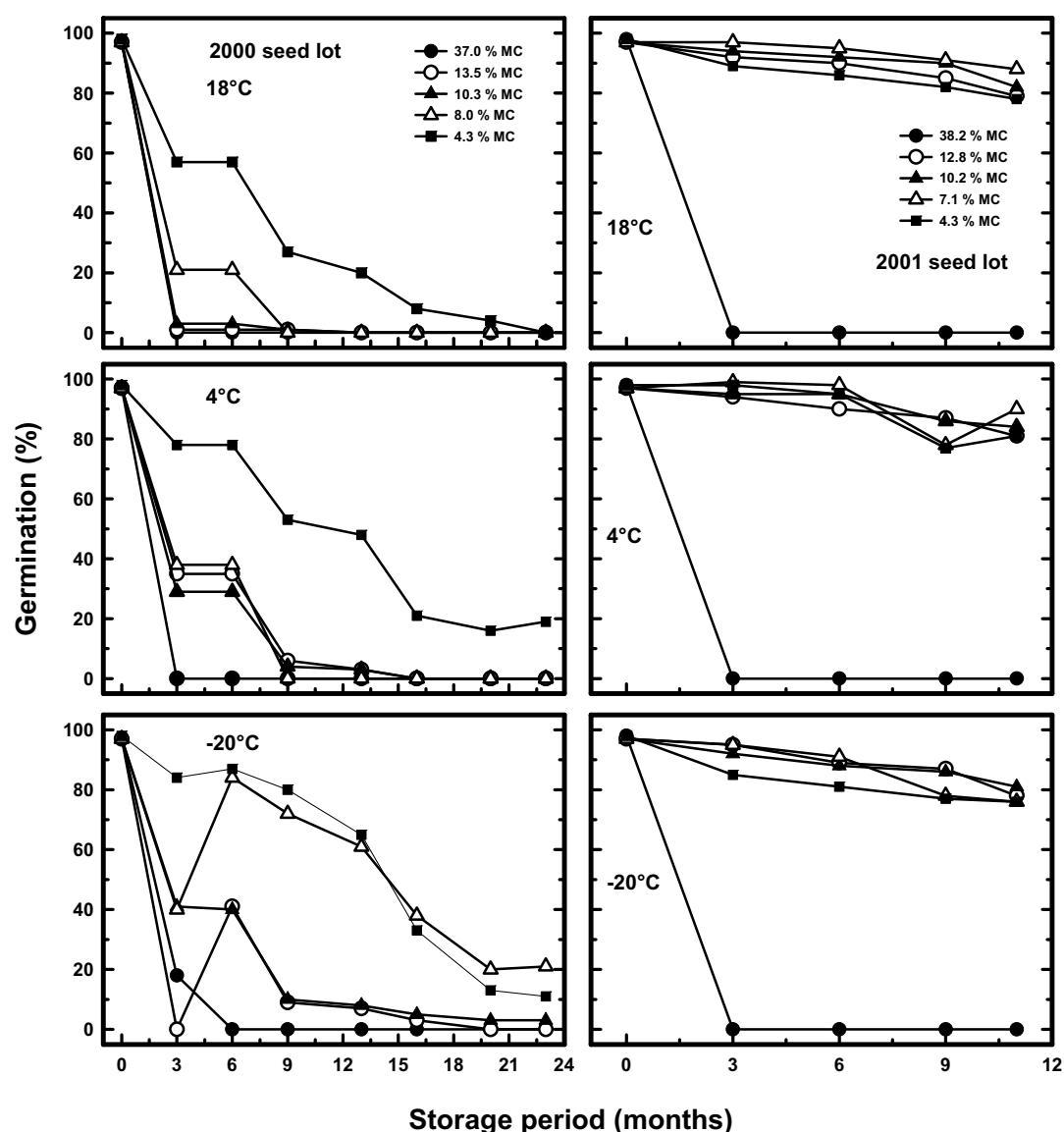


Figure 1. Germination of *A. colubrina* seeds after storage for 23 months (2000 seed lot) and 11 months (2001 seed lot that was not treated with NaOCl).

The protocol is simple and easy to implement. It has been translated to allow studies of other species. For future activities, it is important that many other species be investigated. Establishing a network on recalcitrant seeds would continue to benefit the exchange of information and the publication of results for all the participating countries.

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Desiccation and storage of lucuma, *Pouteria macrophylla* seeds

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Abstract

Pouteria macrophylla seeds from Bolivia have been investigated for their tolerance to desiccation and storage. The maximum weight of the fruits was three times their minimum dry weight of ca. 41 g, while the big seeds weigh about five times compare to the minimum weight of 3.5 g of small seeds. Seeds completed germination over 60 days. While 73% germination was retained after drying seeds to 32% MC, no germination occurred at 19% MC, indicating the desiccation limit for these seeds. These results showed that *P. macrophylla* seeds were desiccation sensitive and that these seeds seemed to require higher temperature of $\geq 30^{\circ}\text{C}$.

Introduction

Pouteria macrophylla (Lam) Eyma, belongs to the Sapotaceae family, with synonyms like *Chrysophyllum macrophyllum* Lam., *Lucuma rivicoa* (Gaertn.) and *Vitellaria rivicoa* (Gaertn.) Radlk.. It is also called Canistel (English), Jaune d'oeuf (French Guayana), yema de huevo (Spanish), Utitiriba (Brazil) (FAO 1986), and Lucuma in Bolivia. It is a small to medium forest tree up to 20–25 m high with a dense crown. *P. macrophylla* is a common species that thrives in moist tropical and subtropical regions of Bolivia. It also naturally occurs in the tropical region of Peru and the Amazonian region of Brazil (Cavalcante 1988). In Bolivia, according to Killeen (1993) it occurs in the northern region of Santa Cruz City, but can also be found in the tropical low land region of Bení, La Paz and Cochabamba. *P. macrophylla* grows in secondary forests, near the disturbed areas of rural homes, in the moist low land region, generally in deeply and well-drained soils, at altitudes between 315 and 450 m above sea level. It grows well in areas with annual rainfall between 1200 and 2800 mm and a mean temperature of 24°C . The trunk is straight, and attains up to 50 cm in diameter, with

deep crevices near their base. The wood is soft and gray-yellow. It is used in construction and in door and window frames and agricultural implements. The bark exudes white latex when it is cut. Branches in juvenile trees are ascending, becoming more horizontal at maturity. The leaves are simple alternated without stipules, with petioles of 2.5–3.5 cm long and blades that are oblong lanceolate and 10–20 cm long times 4–8 cm wide.

In Bolivia, especially in Santa Cruz, flowering of *P. macrophylla* occurs from Sept to Nov, the last flowers persisting until Jan. The flowers are small and organized in fascicles, with a greenish corolla of up to 10 mm long. The tree fruits from Feb to Mar. The fruits are ovoid and up to 6 cm diameter. When there is a single seed per fruit, the seeds are also ovoid and when there are two or three seeds per fruit, the seeds are flat. The seeds are covered with a dark brown hard testa embedded in a starchy yellow pulp. *P. macrophylla* reproduces by seed, germinating start about 50 to 60 days after sowing (FAO 1986). We have selected the lucuma species because of its great interest in agroforestry systems, its potential use for reforestation in secondary forests, and for its edible fruits. Compared with other native species, it is rapid growing under good conditions (1–1.5 m yr⁻¹). The starchy mesocarp of the fresh fruits has an agreeable and generally sweet flavour, which is the part directly consumed by people mainly in rural areas. The objective of this work was to study the desiccation behaviour of *P. macrophylla* seeds.

Materials and methods

Collection of fruits and desiccation of seeds

Fruits were directly collected from trees in Buena Vista, 120 km North from Santa Cruz City in a low land region of Bolivia, near the Andean mountains. Its climate is subtropical, with 1400 mm annual rainfall and 24°C mean annual temperature. Mature fruits of *P. macrophylla* were collected 16–18 Feb 2001, from 20 trees within the dense young secondary forest, which had been re-established from natural regeneration. The fruits were transported in two permeable bags of 10 kg each to Santa Cruz City the following day. The collected fruits were put into bamboo sieves in shade, for 2–3 days to avoid an excessive humidity loss.

The fruits were manually and tediously processed, by removing the pulp from seeds. Seeds were then washed with tap water, and immediately soaked in 10% sodium hypochlorite for 10 min, as indicated in the protocol (IPGRI/DFSC 1999, 2000). Soon after preparation, seeds were sent to Cochabamba for laboratory experiments.

Moisture content and germination of seeds

Seed samples were dried down to target moisture contents of 40, 35, 30 and 20%, using silica gel. To determine the moisture content of seeds, five replicates of 20 seeds each were weighed before and after drying at 103°C for 17 h. The moisture content was then calculated using the formula: $(IW-FW)/IW \times 100$, where IW=initial weight and FW=final weight.

The germination of seeds was designed in a random block. Seeds were sown in sterilized sand into rectangular polystyrene boxes with cover [30×25 cm (base)×8 cm (height)]. The boxes were then put in a room at constant 28°C and 60% relative humidity. In a second trial, *P. macrophylla* seeds were incubated in fluctuating temperatures between 15 and 38°C, for germination. Artificial light was provided for 8 h each day.

Results

Initial characteristics

There was large variation in weights of the fruits and seeds of *P. macrophylla*. The maximum weight of the fruits was three times their minimum weight, whilst the maximum compared to the minimum weight for the seeds was about five times (Table 1). These results are based on 100 fruits and their seeds.

Table 1. Fruit and seed characteristics of *P. macrophylla*.

| Parameter | Fruits | | | Seeds | | |
|-----------|------------|-------------|------------|------------|-------------|------------|
| | Weight (g) | Length (cm) | Width (cm) | Weight (g) | Length (cm) | Width (cm) |
| Maximum | 126.06 | 7.01 | 5.54 | 15.14 | 4.21 | 2.72 |
| Minimum | 40.85 | 3.08 | 2.68 | 3.46 | 2.07 | 1.68 |
| Average±S | 67±17.2 | 4.07±0.61 | 3.42±0.48 | 9.15±2.5 | 3.06±0.33 | 2.26±0.2 |
| D | | | | 1 | | 1 |
| CV (%) | 26 | 15 | 14 | 27 | 11 | 9 |

Moisture content and germination

Seeds were desiccated to various target MCs and then tested for their germination capacity at 28°C. The time to complete all germination was 60 days. Whilst germination of 73% was obtained after drying to 32% MC, no germination occurred at 19% MC, indicating the desiccation limit for these seeds (Table 2).

In a second test, *P. macrophylla* seeds were incubated at 15–38°C for germination. Seeds at all tested moisture levels germinated better at 15–38°C, which was out side the germination chamber, than at constant 28°C in the germination chamber (Table 2), showing that *P. macrophylla* seeds needed high temperature to germinate.

Table 2. Germination (%) of seeds after drying to four moisture contents and at varying (15–38°C) and constant (28°C) temperatures.

| Treatments | Target MC (%) | Actual MC (%) | Germination (%±SD) | |
|------------|---------------|---------------|--------------------|---------|
| | | | 28°C | 15–38°C |
| A | Initial | 46.32 | 67±4.4 | 78± 2.8 |
| B | 40 | 41 | 82±2.3 | 72±0 |
| C | 35 | 36 | 74±3 | 60±5.7 |
| D | 30 | 32 | 73±3.8 | 46±19.8 |
| E | 20 | 19 | 0 | — |

Discussion

Fruits and seeds of *P. macrophylla* had big variations in shapes and sizes, and also in their weights (Table 1). In a separate test in Cochabamba, the testa was removed and the seeds did not germinate (data not shown). It is thus recommended that the seed testa is not separated or broken prior to the start of the germination tests. Although the mean temperature out side the germination chamber was 26°C, which was close to the 28°C within the chamber, germination of these seeds seemed to require higher temperature of >30°C. Thus, there is still a need to further investigate seed germination temperature, the flowering system and period of fruiting of this species. The seeds tolerated desiccation to ca. 30% MC, but not lower, indicating that *P. macrophylla* seeds are desiccation sensitive.

This is a first work in the Gabriel Rene Moreno University, related to a systematic study of the collection and storage of forest seeds, using the IPGRI/DFSC protocol. We expect to set up new trials on other species, using this protocol as a principal guide.

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Desiccation, storage and germination of *Genipa americana* seeds

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Abstract

Germination temperature dependency, tolerance to desiccation and sensitivity to exposure to low and sub-zero temperatures were investigated on *Genipa americana* seeds. The optimum temperature for germination of fresh seeds was found to be 30°C. The critical moisture content (seed viability reduced to 50%) for the seeds was between 9 and 6%. Germination capacity was maintained for 12 months during storage at 5, 10 and 15°C, when seeds were at *ca.* 11% moisture content. A drastic reduction of seed viability occurred after -20°C exposure. These results suggest that *Genipa americana* seeds have intermediate behaviour.

Introduction

Genipa americana L. (local name: jenipapo) is a member of the Rubiaceae family that occurs in humid Brazilian ecosystems. The wood of the species is used for many purposes. The Indians use the blue juice made from immature fruits to dye their skin. The mature fruits are edible and used to prepare ice cream, pudding, juice, wine and liquor (Villachica *et al.* 1996). The seed has a flat and irregular, sometime rectangular, shape. The seed coat is thin and yellowish-brown and the endosperm is yellowish-white.

Classifying these seeds into the correct storage behaviour and establishing appropriate conditions for germplasm conservation are important for the long-term conservation of *G. americana*. The seeds have been found to have a short lifespan, however, there are conflicting reports of actual seed storage category with suggestions of both recalcitrant and intermediate seed storage behaviour (Lorenzi 1992; Carvalho and Nascimento 2000). In this study, the responses to germination temperature, desiccation and storage conditions were investigated.

Materials and methods

Seed collection and extraction

Fruits were collected in 1998 from three trees 1 km apart, in the savannah vegetation at Mangabeira farm (access road BR 080) in the Mato Seco region, state of Goiás. The seeds were extracted by hand. After removing the fleshy pulp by rubbing the fruits in a sieve, the seeds were washed in tap water. The same day, moisture content determinations and desiccation trials were initiated.

Fruit and seed weights were determined for 100 individual fruits and their seeds. Initial moisture content was determined for 100 individual seeds and for five replicates of each of five whole seeds, 20 isolated embryonic axes, and 20 isolated endosperms plus seed coats.

Effect of temperature on seed germination

Four replicates of 25 fresh (nondried) seeds were germinated on moistened paper towel, at a range of constant temperatures between 5 and 40°C with 12 h light per day. Samples of seeds were dried at room temperature ($25\pm 2^\circ\text{C}$) by mixing with equal amounts of silica gel. Control samples were placed in similar containers with vermiculite instead of silica gel. The desiccation periods were from 0 up to 72 h. After each desiccation period, MC was determined on five replicates of five whole seeds, and germination tests were carried out with four replicates of 25 seeds each, at 30°C with 12 h light per day.

Seed desiccation and response to -20°C

Further samples of ≥ 225 seeds each were dried at room temperature with silica gel, as before, for between 24 and 72 h. After desiccation, moisture content (MC) was determined using five replicates of five whole seeds each and germination tests were carried out with four replicates of 25 seeds, incubating them at 30°C with 12 h light per day. The remaining 100 dried seeds were placed at -20°C for 24 h before sowing for germination at 30°C, as described above.

Storage trials

Seeds treated with fungicide were dried to four different moisture contents. Sub-samples were then mixed with vermiculite, sealed in impermeable bags, and stored at 5, 10 and 15°C for 2, 4, 6, 8, 10 and 12 months. Seeds were regularly taken to test their germination capacity as described above.

Results

Mean fruit and seed weights were 175.5 ± 53.2 g and 0.09 ± 0.01 g, respectively. The initial MC of whole seeds was ca. 44–46% fresh weight (Table 1). For seed tissues, the initial MC was higher for embryonic axes (76%) compared with seed coats and endosperms (37%; Table 1).

Table 1. Initial moisture contents of seeds and seed tissues

| Material | MC \pm s.d. (%) |
|---|-------------------|
| Mean of 100 individual seeds | 43.8 \pm 2.60 |
| Mean of five replicates of five whole seeds | 46.0 \pm 5.31 |
| Mean of five replicates of 20 embryonic axes | 76.5 \pm 0.69 |
| Mean of five replicates of 20 endosperms plus seed coat | 38.0 \pm 0.69 |

Effect of temperature on seed germination

Maximum germination was achieved at temperatures between 15 and 30°C, while no seeds germinated at 5 and 40°C (Fig. 1). At 10°C, radicles protruded from 97% of the seeds, but only 31% developed into seedlings; at 15, 20, 25 and 30°C, normal seedlings developed within 84, 46, 26 and 22 days, respectively. Radicles of germinated seeds at 35°C were necrosed.

Desiccation trials

High germination percentages were maintained for seeds dried to moisture contents between 47 and 19% (Table 2). A slight decrease in viability (to 78%) was observed for seeds dried to 9% MC, whereas viability was reduced to 29–43% germination in seeds dried to approximately 7% MC.

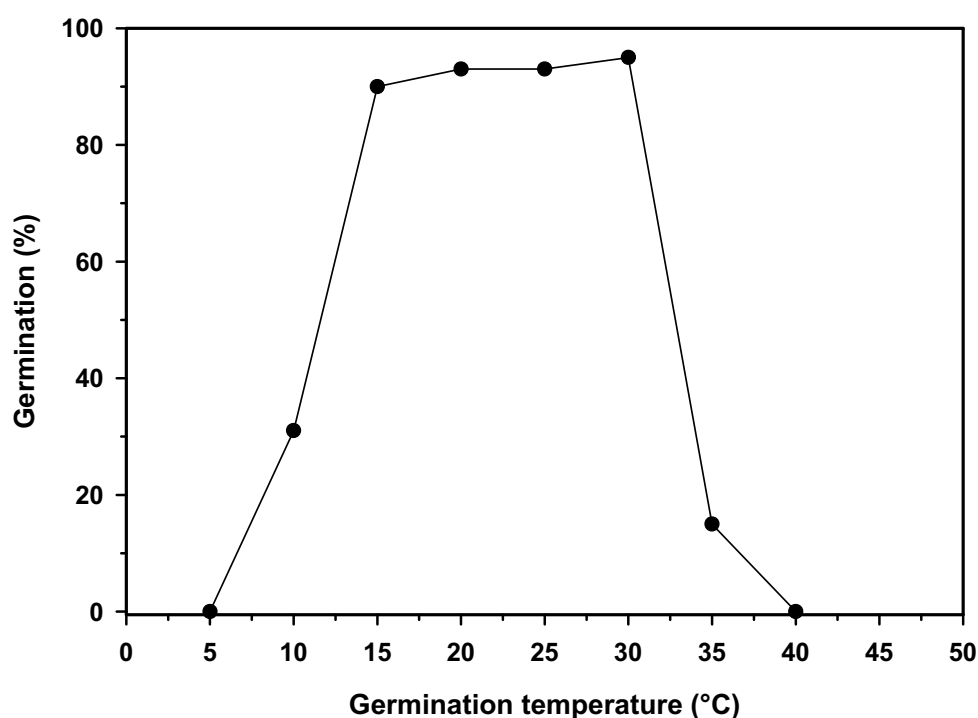


Figure 1. Effect of temperature on the germination of *G. americana* seeds. Each data point represents mean of four replicates of 25 seeds.

Table 2. Effect of desiccation (MC%) on seed germination capacity (Germ.%)

| Desiccation (h) | Controls (vermiculite) | | Desiccated seeds (silica gel) | |
|-----------------|------------------------|-----------|-------------------------------|-----------|
| | MC±s.d. (%) | Germ. (%) | MC±s.d. (%) | Germ. (%) |
| 0 | 56.48±1.46 | 96 | — | — |
| 3 | 51.48±1.12 | 99 | 47.43±0.90 | 91 |
| 5 | 53.88± 0.65 | 97 | 45.41±1.29 | 87 |
| 8 | 53.33±2.87 | 97 | 43.95±2.16 | 93 |
| 12 | 54.01±0.24 | 94 | 36.79±1.75 | 97 |
| 16 | 53.12±1.08 | 100 | 34.84±1.79 | 97 |
| 20 | 52.61±0.79 | 99 | 25.20±2.29 | 96 |
| 24 | 50.84±1.70 | 99 | 19.23±2.51 | 92 |
| 36 | 54.49±1.07 | 99 | 9.34±1.03 | 78 |
| 48 | 53.12 ±1.39 | 96 | 6.88±0.24 | 43 |
| 72 | 48.31±0.45 | 100 | 6.72±0.25 | 29 |

Effect of desiccation and exposure to -20°C on seed viability

Low germination percentages (between 0 and 15%) were obtained for seeds at all moisture contents placed at -20°C for 24 h (Table 3). The best result of 15% germination was observed for seeds dried to 8.6% MC, while viability was no more than 3–4% at moisture contents above or below this level.

Table 3. Effect of desiccation and exposure to -20°C for 24 h on seed viability

| Desiccation (h) | MC (%) | Germination (%) | |
|-----------------|-----------------|------------------------------|-------------------------------------|
| | | Before -20°C | After 24 h at -20°C |
| 0 | 51.5 \pm 1.10 | 99 | — |
| 24 | 13.2 \pm 2.20 | 66 | 3 |
| 28 | 8.6 \pm 0.23 | 61 | 15 |
| 48 | 7.1 \pm 0.24 | 41 | 4 |
| 52 | 6.5 \pm 0.65 | 31 | 1 |
| 72 | 6.3 \pm 0.09 | 33 | 0 |

Storage trials

Seed viability decreased during storage at 5, 10, and 15°C (Fig. 2). Rate of loss of viability appeared to be fastest for seeds at 38% MC. However, for seeds at both 42% and 38% MC there was no germination after 12 months storage at 5, 10 or 15°C . High levels of germination ($>80\%$) were maintained in seeds dried to 11% MC and stored for 12 months at 5, 10 or 15°C .

Discussion

Drying seeds of *G. americana* resulted in a decrease in germination percentage, the critical MC being somewhere between 9 and 6% fresh weight (Tables 2 and 3). Although the seeds showed sensitivity to -20°C (Table 3), germinability was maintained during 12 months storage at 5, 10 and 15°C , when seeds were stored with ca. 11% moisture content. These results show that *G. americana* seeds are not recalcitrant and may have intermediate storage behaviour. Unfortunately, the survival of seedlings in the greenhouse was compromised by *Fusarium oxysporum* contamination.

A preliminary test showed that regeneration of *G. americana* embryonic axes after desiccation and exposure at -196°C was unaffected by bacteria contamination. Therefore, it is suggested to develop a cryopreservation protocol for embryonic axes of this species.

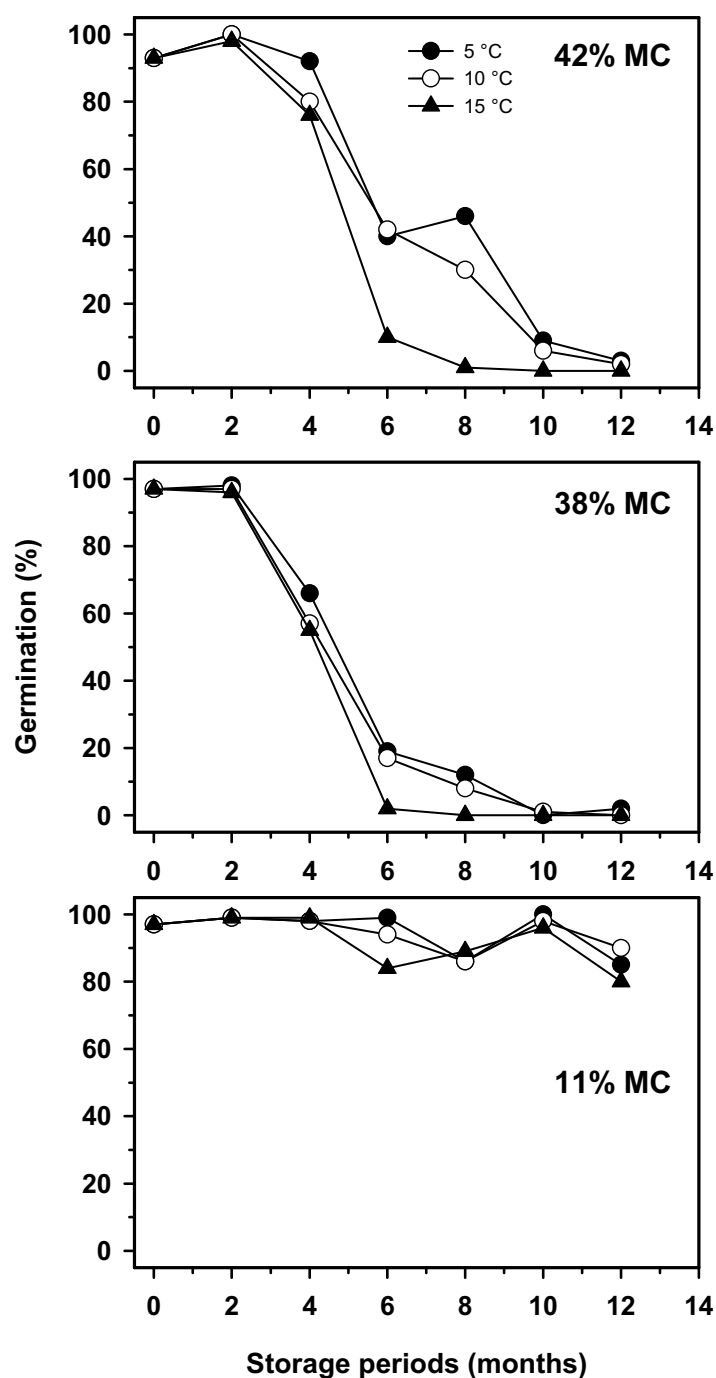


Figure 2. Germination response of *G. americana* seeds dried to 42, 38 and 11% MC and stored at 5, 10 and 15°C for 12 months.

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Desiccation, storage and germination of *Hancornia speciosa* seeds

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Abstract

When seeds of *Hancornia speciosa* were incubated at 5 up to 40°C, they did not germinate at 5°C, while maximum germination was obtained at 10°C, indicating chilling sensitivity below this temperature in these seeds. The highest (100%) germination was obtained at 25°C. Seeds could be partially desiccated to 33% MC without significant reduction in germination and be further dried to 9% MC with great decrease in germination capacity. However, seedling vigour was affected by desiccation, when seeds were dried to or below 26% MC. In the storage experiments, seed viability was not maintained for longer than 2 months at 5 and 10°C. *H. speciosa* seed responses to dehydration and storage at low temperatures confirmed its classification as a recalcitrant species.

Introduction

Hancornia speciosa Gomez (Apocynaceae), named locally as mangaba, mangabeira, occurs in low and high frequency in semi-arid and savannah regions of Brazil. The species produces an edible fruit, which can be consumed *in natura* or used to prepare ice cream, pudding, juice, jam, wine, vinegar and liquor. The seed is a flat and irregular discoid with a central hilum. The seed coat is thin and yellowish-brown and the endosperm is white (FAO 1986; Lorenzi 1992). The seed classified as recalcitrant has a short lifespan (Oliveira and Valio 1992).

Recently, the species has been included in breeding programmes, due to its nutritional and commercial values. The establishment of conditions for germplasm conservation becomes a priority to meet breeders' demands.

Materials and methods

Seed collection and extraction

Fruits were collected in Oct and Nov 1996, and in Nov 1997 and 1998, each harvest composing a seed lot. As shown in Table 1, collections were made from three different locations to obtain enough seeds in 1996 and from only one location in the other years. The collection was made from the ground in grazing-lands in each of the locations.

The seeds were extracted from fruits by hand. After removing the fleshy pulp by rubbing the fruits in a sieve, the seeds were washed in tap water. Moisture content determination and desiccation trials were initiated the same day of seed processing.

Initial tests

Fruit and seed weights were determined on 100 individuals. Seeds of lots 1 and 4 were used to determine seed weights, and fruit weights were measured using lot 4. Initial moisture contents were measured on individual seeds (1×100 seeds) of lots 1 and 4, and on samples of whole seeds (5×3 to 5 seeds) of lots 1, 2, 3 and 5. Seed components were also used to measure moisture contents of a sample of 10 excised embryonic axes and endosperms from lots 1 and 4.

Table 1. Seed lots used in the trials

| Collection | Seed lot | Provenance |
|------------|----------|--|
| 1996 | 1 | 54.5 km from Brasília (route to Unaí) |
| | 2 | Mozondó farm, near Maranhão river, between District Federal and the State of Goiás |
| | 3 | Vãozinho de dentro farm, 55 km from São João da Aliança municipality, State of Goiás |
| 1997 | 4 | Mozondó farm, near Maranhão river, between District Federal and the State of Goiás |
| 1998 | 5 | Near to Mutuca farm, 60 km from São João da Aliança municipality, State of Goiás |

Effect of temperature on seed germination

Germination tests were performed by placing two replicates of 25 seeds on a layer of cotton wool moistened with distilled water, over a

range of constant temperatures between 5 and 40°C (lots 1 and 2), and a photoperiod of 12 h light per day.

Desiccation trials

Seeds were desiccated, mixed with silica gel (4 g silica/1 g seed) at room temperature ($25\pm 2^\circ\text{C}$), for 0 and 100 h (lot 1), for 0 and 48 h (lot 2), and for 0 up to 92 h (lot 4). After each desiccation period, moisture content was determined on five replicates of five seeds (lots 1 and 2) and on 10 individual seeds (lot 4). Germination tests were carried out using two replicates of 25 seeds of lots 1 and 2, and 4 replicates of 25 seeds of lot 4, at a constant temperature of 25°C , and a photoperiod of 12 h light per day.

In a separate trial, seeds from lot 5 were desiccated, mixing them with an equal amount of silica gel. Controls were placed in similar containers with vermiculite in place of the silica gel. Dehydration periods of 0 up to 63 h were determined in line with the results of preliminary desiccation trials. After each desiccation period, moisture content was determined with five replicates of five whole seeds, and germination tests were carried out using four replicates of 25 seeds, at a constant temperature of 25°C and with a photoperiod of 12 h light per day.

Storage trials

After desiccation and fungicide application, samples of seeds of lot 5 were mixed with vermiculite and sealed in impermeable bags. Seeds desiccated to 52.5, 50.7 and 47.7% MC were stored at 5°C , and seeds with 49.0 and 38.5% MC were stored at 10°C , storage at both temperatures lasted for 2, 6 and 12 months.

Results

Initial tests

Fruit weights varied greatly within the same population, whereas there was a smaller variation in seed weights (Table 2). Initial moisture contents were high, around 50% for all seed lots (Table 3).

Effect of temperature on germination

Of all tested temperatures, *H. speciosa* seeds did not germinate at 5°C, while maximum germination was obtained at 10°C. High germination percentages of 88 to 100% were obtained for seeds incubated at 10 to 30°C, above which temperature viability declined to 58 and 4% (Fig. 1). However, it has been observed that seeds initiated germination at 10, 15, 35 and 40°C, with only radicle protrusion but not normal development of seedlings. This occurred only with seeds germinating at 20, 25 and 30°C.

Table 2. Mean weights of 100 individual seeds and fruits from lots 1 and 4

| Material | Weight (g±sd) |
|---------------------|---------------|
| 100 seeds (lot 1) | 0.228±0.052 |
| 100 seeds (lot 4) | 0.184±0.063 |
| 100 fruits (lot 4) | 42.188±18.192 |

Table 3. Mean initial moisture contents of seeds and seed components

| Material | Moisture content±sd (%) |
|---------------------------------------|-------------------------|
| 100 individual seeds (lot 1) | 51.13±3.61 |
| 100 individual seeds (lot 4) | 55.69±8.23 |
| 5×5 whole seeds (lot 1) | 52.90±1.54 |
| 5×3 whole seeds (lot 2) | 51.53±0.99 |
| 5×3 whole seeds (lot 3) | 53.74±1.80 |
| 5×5 whole seeds (lot 5) | 50.63±1.10 |
| 10 individual embryonic axes (lot 1) | 78.08±3.51 |
| 10 individual endosperms (lot 1) | 48.68±4.60 |
| 10 individual embryonic axes (lot 4) | 77.53±4.22 |
| 10 individual endosperms (lot 4) | 45.57±6.33 |

Desiccation trial

Table 4 and Figure 2 present the effect of desiccation of *H. speciosa* seeds from different lots. Germination percentage decreased after drying seeds to ca. 25% MC, and no seed germinated at 7% MC and below. The critical moisture content for the onset of viability loss seemed to be around 30%. Although some seeds germinated at lower moisture contents, reduced vigour was observed in seedlings from seeds dried to 25% MC and below (see Table 4). High moisture content was maintained in the control seeds in vermiculite, which also germinated over 80% on average.

Storage trial

Storage at 5°C led to approximately 50% germination or less, whereas more than 70% were obtained at 10°C after 2 months storage. Only 5% of seeds with 46% MC germinated after 6 months at 10°C. No other seed germinated after storage for 12 months, irrespective of conditions (see Table 5).

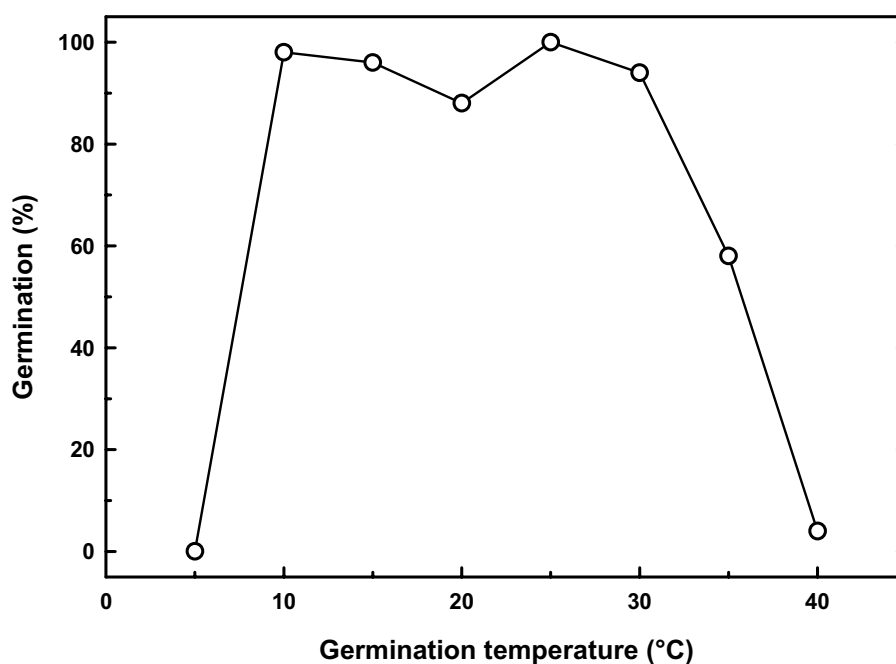


Figure 1. Effect of germination temperature on *H. speciosa* fresh seeds from lot 1.

Table 4. Effect of desiccation using an equal amount of silica gel (g g^{-1} seed), on the viability of seeds from lot 5

| Desiccation Period (h) | Vermiculite (control) | | Silica gel (drying) | | |
|---------------------------|-----------------------|-----------------|---------------------|-----------------|---------------------------------|
| | MC \pm sd (%) | Germination (%) | MC \pm sd (%) | Germination (%) | Observations on seedling vigour |
| 0 | 50.6 \pm 1.10 | 80 | — | — | — |
| 4 | 48.8 \pm 0.88 | 88 | 46.7 \pm 1.75 | 86 | — |
| 12 | 45.3 \pm 1.98 | 90 | 32.9 \pm 1.95 | 93 | — |
| 20 | 47.1 \pm 0.85 | 86 | 25.6 \pm 2.44 | 75 | Reduced |
| 24 | 47.1 \pm 1.99 | 89 | 19.2 \pm 1.40 | 62 | Reduced |
| 28 | 45.5 \pm 2.20 | 85 | 18.9 \pm 2.36 | 70 | Reduced |
| 44 | 45.8 \pm 2.91 | 83 | 9.1 \pm 1.27 | 23 | Reduced |
| 51 | 46.9 \pm 1.23 | 78 | 7.2 \pm 0.84 | 0 | — |
| 68 | 44.7 \pm 3.88 | 81 | 5.9 \pm 0.37 | 0 | — |

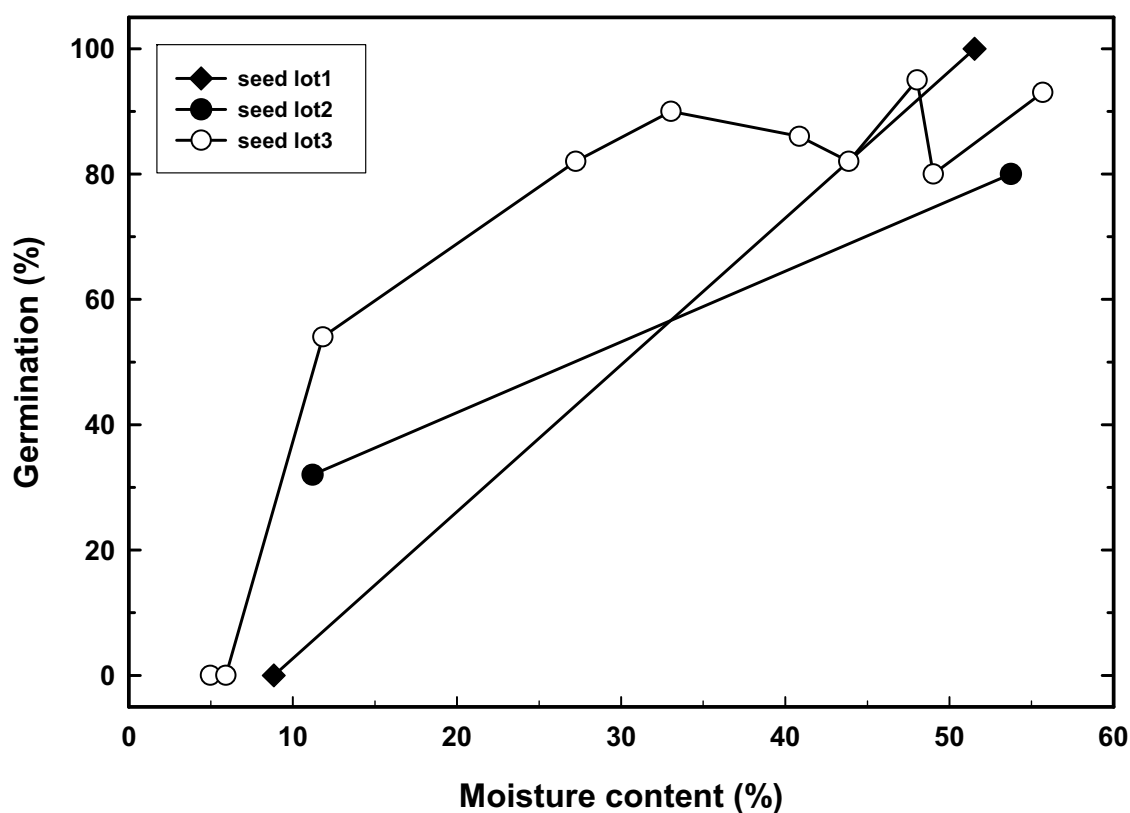


Figure 2. Relationship between moisture content and germination of *H. speciosa* seeds.

Table 5. Seed germination (G) response to storage conditions at 5 and 10°C for 12 months

| Storage temp. | MC±sd (%) | Initial G (%) | Storage period | | | | |
|---------------|------------|---------------|----------------|-------------|-------|-------------|-------|
| | | | 2 months | 6 months | | 12 months | |
| | | | G (%) | MC (%) | G (%) | MC (%) | G (%) |
| 5°C | 52.54±3.86 | 80 | 40 | 9.52± 0.27 | 0 | 38.70± 1.14 | 0 |
| | 51.12±3.86 | 84 | 58 | 9.52± 0.27 | 0 | 36.88± 0.99 | 0 |
| | 47.69±2.63 | 86 | 36 | 39.55± 0.93 | 0 | 13.90± 1.47 | 0 |
| 10°C | 48.99±2.97 | 84 | 90 | 46.27± 3.12 | 5 | 45.46± 2.87 | 0 |
| | 38.46±2.91 | 82 | 73 | 40.29± 3.88 | 0 | 40.76± 3.61 | 0 |

Discussion

Seeds of *H. speciosa* did not germinate at 5°C, while maximum germination was obtained at 10°C, indicating chilling sensitivity in these seed species. Seeds could be partially desiccated to ca. 30% MC without significant reduction in germination and be further dried to 9% MC with great decrease in germination capacity. However, seedling vigour was affected by desiccation, when seeds were dried to or below 26% MC. In the storage experiments, seed viability was not maintained for longer than 2 months at 5 and 10°C.

During germination, fungal infection compromised the capacity of seeds. The principal identified fungi were *Fusarium oxysporum*, *Penicillium* sp, *Periconia* sp, *Rhizopus* sp and *Torula* sp. However, a preliminary test showed that seed tissues were not affected by bacteria contamination during regeneration of embryonic axes after desiccation or desiccation followed by exposure at -196°C. It is therefore suggested to develop a cryopreservation protocol for the *ex situ* conservation of this species.

Conclusion

Hancornia speciosa seeds cannot withstand desiccation below 30% MC, which should be avoided. Storage conditions, including temperatures above 10°C need to be further investigated. On the basis of the present results, it must be recommended to avoid germination or storage below 10°C.

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Desiccation and storage of *Cariniana pyriformis* seeds

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Abstract

Cariniana pyriformis seeds from Colombia have been desiccated and stored at different conditions. The results showed that the initial germination of seeds at 12.9% MC was 42%. When these seeds were dried down to 3% MC, germination increased to 54%. Seeds with different moisture contents maintained viability after storage at different temperatures. *C. pyriformis* seeds with 12.8% MC retained a constant 35% germination after storage at 18°C for 1 and 2.5 months. The lowest germination capacity was around 7% for seeds stored at 30°C. Although the storage period was short, it seems clear that these seeds maybe storable for the long term in dry conditions and at low (cool) temperatures.

Introduction

Cariniana pyriformis Miers, or abarco belongs to the Lecythidaceae (Brazil-nut family). It is naturally distributed in Panama and Colombia. In Colombia the species grows in the three mountain ranges that extend through the country. Other species within the same genus are found in Costa Rica, Brazil, Peru, Paraguay, Bolivia and Trinidad – Tobago. It is also called Colombian mahogany, and its timber is highly valued in Colombia because of its good finish and multiple uses. The timber is very durable and resistant to fungi and insects. Being easy to work, it is used for construction, furniture, pencils and boards. The exploitation of its timber has led to genetic erosion to such an extent that it is now considered endangered. The species is qualified for a vulnerable status on the IUCN Red List of Threatened Species (IUCN 2002). It has become necessary to take action to conserve the species and its genetic resources, and in addition, to find improved methods for its propagation.

The abarco reaches up to 30–40 m in height and grows to a diameter of up to 2 m. The trunk is fissured and dark brown, with

bark flaking off in large patches. The crown is umbrella-shaped. The species is deciduous, i.e. the leaves are shed at the beginning of the cold or dry season. Flowers are white, hermaphroditic, with 5–6 petals and 5–6 sepals and borne in terminal or axillary panicles. The androecium has numerous fertile stamens that are fused with the petals. Ovary inferior is composed of three locules each containing several ovules. The fruit is woody, pear-shaped or oval, opening with a lid. The species propagation is possible vegetatively as well as by seed. Cuttings taken from the middle part of the crown are used for vegetative propagation, rooting to about 75%. When propagated by seeds, a germination of about 50% is easily achieved. The species is a semiheliophyte growing in primary and late secondary forests. It is planted at a distance of 4×4 m to achieve an average growth of 6 m³ ha⁻¹ y⁻¹ (Lastra 1971).

In Colombia, flowering takes place in Nov–Dec, while fruiting is in Jan–Mar. However, with great variations in fruit production in this species, it can be difficult to predict when collection should take place. Seeds are mature when the fruit has turned dark brown and the lid (operculum) begins to come loose. Six seed production areas have been identified in Colombia, all in the northern part of the country. When the capsules open, the seeds are widely dispersed making it necessary to collect the fruits before they open. However, collection from the tree is difficult because of the high height of trees and the fruits are situated at the end of the branches. Thus, fresh fruits are often collected from the ground.

Materials and methods

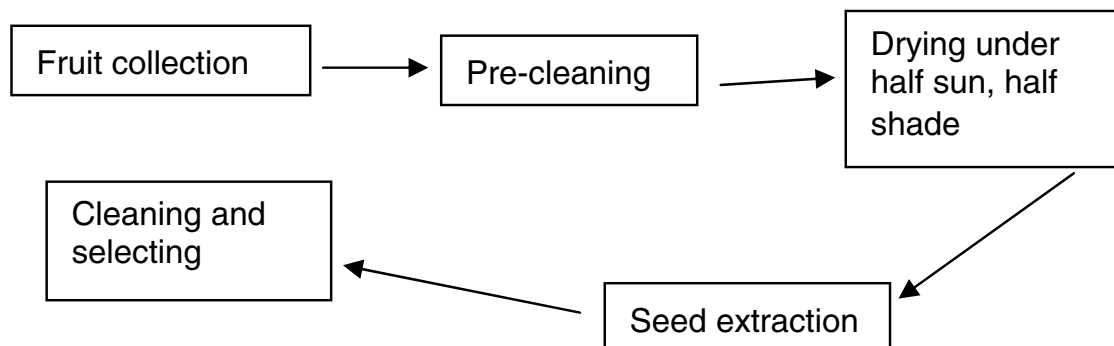
Seed collection and processing

Fruits were collected from an identified source of one ha within a 600 ha forest dominated by *C. pyriformis*. The ground under the 20 trees was carefully cleaned before collecting fruits on the 13 Nov 2001. The fruits were packed in linen bags and transported to the laboratory by air within two days of collection. The containers were perforated to allow exchange of air during transport and temporary storage. Temperatures during (air) transport were estimated to fluctuate between 8 and 25°C.

Fruits were processed by half shade drying until the capsules opened to release seeds. The fruits that did not open were considered

as immature and discarded. As far as it was possible, fruits and seeds were protected from excessive temperatures and mechanical damage.

Diagram of seed processing:



After extraction, all damaged or infected seeds were discarded. Finally the seeds were treated with 1% NaOCl for 50 seconds and then rinsed with water. Processed seeds were then kept in hermetically closed containers at ambient temperature (8–14°C) for 15 days, the delay caused by lack of staff to carry out experiments immediately.

Seed characteristics were examined and seed weight was determined on eight replicates of 100 seeds. The unit for all trials was the winged seed (including the testa, which is difficult to remove without damaging the seed). Initial determinations of seed purity and seed weight were carried out according to ISTA rules (1999)

Desiccation and germination

Seeds were mixed with silica gel to desiccate in plastic bags. The controls were put in plastic bags without silica gel. After desiccation, seed samples were weighed before and after drying in oven at 103°C for 17 h. The moisture content was then calculated as a percentage of the fresh weight (IPGRI/DFSC 1999, 2000).

Four replicates of 25 seeds were used for each germination tests. Seeds were sown in soil and river sand (1:1) at a photoperiod of 12/12 hours light/dark and at constant 25°C. Germination data were analyzed for their differences using Duncan's probability test at 5%.

Storage trials

Seeds dried to different moisture contents were sealed in aluminium bags and stored at 4, 18 and 30°C and at ambient temperature of an average 12°C (between 8 and 14°C). Controls at their initial MC were divided into four samples for storage in closed containers at ambient temperature (12°C), 4, 18 and 30°C.

Results and discussion

Initial trials

The mean weight of *C. pyramidalis* fruit was 100 g and there were on average 15 seeds per fruit. After processing, seed purity was determined to be 99.5% (Table 1). There were 8568 seeds per kg, making a thousand seed weight (TSW) about 116.7 g, which is within the range of that found in previous studies (Betancur and Raigosa 1973).

Table 1. Initial characteristics of *C. pyramidalis* fruits and seeds

| Parameters | Values |
|----------------------|-----------|
| Mean fruit weight | 101 g |
| No. seeds/kg fruits | 150 seeds |
| Purity | 99.4% |
| Thousand seed weight | 116.7 g |
| Number of seeds/kg | 8568 |
| Initial MC | 12.92% |

The winged seeds were black when moist and coffee-coloured when dried. Their coat consisted of a layer of testa, which was hard and dry, and of a thin and semi-transparent tegument. Seeds had low initial moisture content of 8.3% (Table 2), although immediately after leaving in the shade, mature fruits had between 10 and 20% MC. Green fruits that were not used in these trials had a higher initial moisture content (36%). The 8.3% MC increased to 12.9% MC (Table 1) after the NaOCl treatment, which was the initial moisture content for all experiments. Seeds with 12.9% initial MC were then dried to 7.8, 6 and 3% MC.

Table 2. Initial moisture content and mean weights of 100 seeds and excised components (embryo and testa)

| | Fresh weight (g) | Dry weight (g) | MC (%) |
|--------|------------------|----------------|----------|
| Seed | 11.67±0.58 | — | 8.3±0.27 |
| Embryo | 9.02 | 8.44 | 6.4 |
| Testa | 3.20 | 2.77 | 13.4 |

Effect of moisture content on germination

Several initial samples were desiccated to different moisture contents (Fig. 1). The moisture content of 7.8 and 6% were reached within about 24 h, while drying seeds down to 3% MC required 4 days (Fig. 1).

C. pyriiformis seeds initially germinated to 42%. However, there was a significant increase of the germination percentage with reduction of the seed moisture content, attaining 53–54% when seeds were dried to 6 and 3% MC (Table 3). The initial germination percentage of ca. 50% is in the range reported by others studies (Rodríguez 2000). The effect of moisture content on germination was significant, after 1 and 2.5 months of storage (Table 3). In general, there was a decrease of germination after storage, but not complete loss of viability. Germination was maintained at 21%, the same level in seeds with 3% MC after 1 and 2.5 months. Seeds with 12.9% MC germinated to 24%, the highest percentage of all conditions after 2.5 months (Table 3).

Storage trials

All seed samples maintained viability after storage at different conditions. Seeds with 6% MC germinated 49% after one month storage at 18°C, but decreased to about 30% in the second month. *C. pyriiformis* seeds with 12.8% MC retained constant 35% germination after storage at 18°C (Table 4). Seed stored at ambient temperature (average 12°C) and at 4°C maintained their viability during the 2.5 months storage. The lowest germination capacity was around 7% for seeds stored at 30°C. Although the storage period was short, it seems clear that these seeds maybe storable for the long term in dry conditions and at low temperatures.

Table 3. Effect of moisture content on seed germination capacity after storage. Data followed by the same letter in column are not significantly different (Duncan probability test at 5%)

| MC (%) | Germination | | | | |
|--------|--------------|------------|-------------|--------------------|--------------------|
| | Onset (days) | End (days) | Initial (%) | 1 month (%) | 2.5 months (%) |
| 12.92 | 13 | 24 | 42 | 26.25 ¹ | 24.50 ¹ |
| 7.89 | 12 | 23 | 28 | 17.50 ² | 14.00 ² |
| 6 | 10 | 21 | 53 | 28.00 ¹ | 19.25 ² |
| 3 | 8 | 20 | 54 | 21.00 ¹ | 21.00 ¹ |

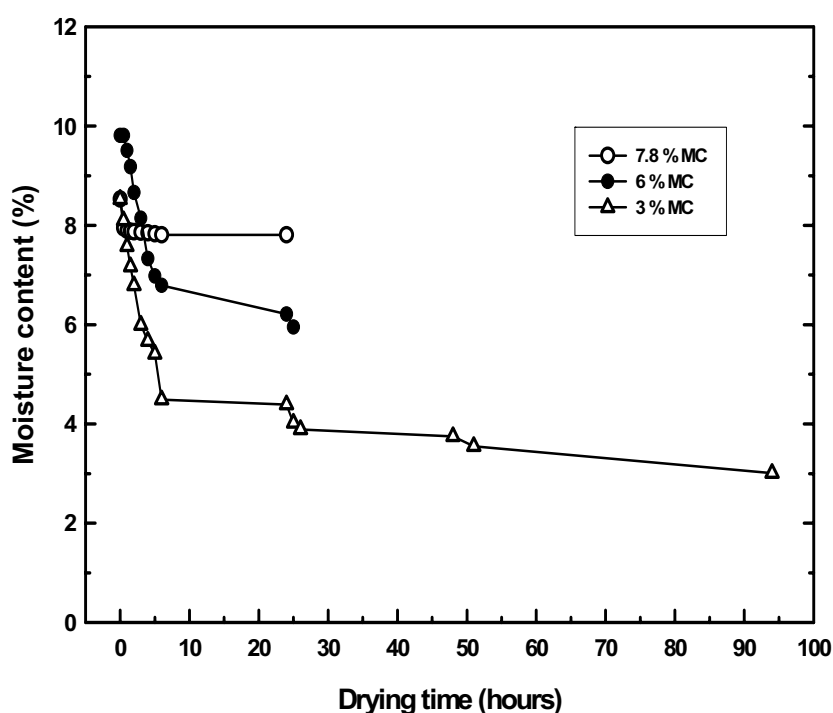


Figure 1. Desiccation time of *C. pyrifomis* seeds to target moisture contents (MC)

Conclusions

C. pyrifomis seeds tolerate desiccation to 3% MC and low (cool) temperature storage. Although the results presented are an evaluation over a short period, they show significant indications as to possible longer-term storage conditions of this species. For short-term storage (at least 3 months), seeds with low moisture content can be stored at a range of temperatures below 20°C. It is recommended that further investigations assess the effects of desiccation and storage conditions on *C. pyrifomis* seeds from other provenances.

Table 4. Germination capacity after storage of *C. pyriformis* seeds at four different conditions of moisture contents and temperatures for 2.5 months. Data followed by the same letter in column are not significantly different (Duncan probability test at 5%)

| MC (%) | Storage (°C) | Germination (%) | |
|--------|---------------|---------------------|---------------------|
| | | 1 month | 2.5 months |
| 3 | 4 | 14.4 ^a | 14.0 ^b |
| | 18 | 35.1 ^{a,b} | 32.0 ^{a,b} |
| | 30 | 14.4 ^b | 7.14 ^a |
| | Ambient (~12) | 21.2 ^{a,b} | 20.9 ^{a,b} |
| 6 | 4 | 14.3 ^b | 14.2 ^a |
| | 18 | 49.0 ^b | 29.3 ^{a,b} |
| | 30 | 14.4 ^b | 7.3 ^a |
| | Ambient (~12) | 35.0 ^{a,b} | 28.5 ^{a,b} |
| 7.8 | 4 | 21.1 ^{a,b} | 21.0 ^{a,b} |
| | 18 | 21.2 ^{a,b} | 14.0 ^b |
| | 30 | 21.2 ^{a,b} | 7.0 ^a |
| | Ambient (~12) | 7.5 ^a | 7.21 ^a |
| 12.8 | 4 | 21.0 ^{a,b} | 20.1 ^{a,b} |
| | 18 | 35.1 ^{a,b} | 35.0 ^a |
| | 30 | 35.2 ^{a,b} | 7.0 ^a |
| | Ambient (~12) | 14.3 ^b | 14.0 ^b |

Acknowledgement

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Desiccation and storage of seeds of *Astronium graveolens* and *Calophyllum brasiliense*, two native species of Costa Rica

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Abstract

Effects of desiccation and storage were investigated for *Astronium graveolens* and *Calophyllum brasiliense* seeds. *A. graveolens* seeds desiccated to 6.6% MC germinated 89%. Seeds with $\leq 8.4\%$ MC stored well for 12 months at -18 , 5 and 15°C , whereas seeds with $\geq 9.9\%$ MC lost viability fast at -18 and 5°C . Fresh *A. graveolens* seeds should therefore be dried below 9% MC and stored at low temperatures. *C. brasiliense* seeds were sensitive to desiccation and low temperatures. Drying seeds with an initial 40% MC and 68% germination to 4.8% MC reduced germination capacity to 4%. Best storage result, 70% germination after three months, was obtained at 15°C for seeds at 32% MC. After 6 months of storage all seeds were dead. It is thus recommended to sow these seeds as soon as possible, or store them for a few months at $>30\%$ MC at 15°C .

Introduction

Costa Rica is a small country, which covers an area of 52 100 km², localized in the middle of Central America between 10°N latitude and 84°W longitude. At the Atlantic coast the rainfall varies from 1500 to 5000 mm, with 2 to 3 dry months (less than 50 mm) per year, while at the Pacific coast the annual rainfall varies from 1500 to 3000 mm, with a drier period of 4 to 6 months. About 23% of Costa Rica is under protection by national parks. *Astronium graveolens* Jacq. and *Calophyllum brasiliense* Cambess., were selected for this study because they are used and grow well in plantations and produce good timber (CATIE 2000). Both species are difficult to reproduce and better methods for seed handling are needed. Flores (1993, 1996) and Sanchez (1995) described these species as being recalcitrant.

Astronium graveolens Jacq. from the Anacardiaceae family, is known as 'ron ron, jocote or jobillo' in Costa Rica. The species grows naturally from Mexico through Central America and south to Brazil, Bolivia and Paraguay. In Costa Rica, it grows from 100 to 600 m above sea level in areas with annual rainfall from 1300 to 3000 mm. The wood is very durable, fairly easy to work and has a specific gravity of 0.85–1.28 g cm⁻³. It is used for furniture, floors, tool handles, cabinets and paper production (DFSC 2000a). The flowers are hermaphrodite, small, with five green-yellow petals; grouped in 10–25 cm long terminal or axillary panicles. In Costa Rica they appear during the drier season between December and March. It is difficult to time the collection of seeds as the fruits mature rapidly, three to four weeks after pollination. The fruits are drupe-like nuts, blue to black at maturity. The single seed is enclosed in a bitter-sweet pulp. The seeds lack endosperm and have high oil content. There are about 18 000 clean seeds per kg (DFSC 2000a).

Calophyllum brasiliense Cambess. from the Clusiaceae family is called 'maría' or 'santa maría' in Costa Rica. The natural range includes southern Mexico, Central America and northern South America. It is also found in the Antilles from Cuba to Jamaica and Trinidad – Tobago. In Costa Rica, this species grows naturally at the Atlantic coast from sea level to 900 m, where annual rainfall reaches over 2500 mm and mean temperature varies between 24 and 28°C. The tree is up to 45 m tall, with straight bole without buttresses and branchless for about two thirds of the height. The bark is thick and contains a yellow-green latex. The wood of *C. brasiliense* is used for both outdoor and indoor construction and is durable in contact with soil and water. The species is andromonoecious, i.e. each tree has both male and bisexual flowers (DFSC 2000b). The trees flower twice a year, between June and July and between November and December. The fruits are green at maturity, but the colour becomes less bright as they ripen. The fruits are, more or less round, berries 2.5 to 3 cm long. Each fruit contains one seed, the pericarp is leathery and dotted with numerous laticifers containing yellow latex. The seed is 1.8–2.3 cm long with large oily cotyledons. There are 415–440 seeds per kg (DFSC 2000b). Despite their uses, little is known on the biology of *A. graveolens* and *C. brasiliense* seeds. We report the results obtained from our investigations on these two species since 1999.

Materials and methods

Seed collection and processing

At the beginning of 1999, CATIE Tree Seed Bank (CTSB) identified seed stands, collected and made seeds of *A. graveolens* and *C. brasiliense* available for desiccation and storage trials for both CTSB and DFSC as the replicating partner. Fruit collection occurred in accordance with the screening protocol (DFSC/IPGRI 1999). *A. graveolens* fruits were collected from eight trees of the Volcán, P. Zeledón (BL095) source in Costa Rica on April 13–14 1999. The fruits were manually collected from the tree by cutting off branches with pruning shears. *C. brasiliense* fruits were collected in the same way in Buenos Aires, Puntarenas (BL096) source in Costa Rica on 15 April 1999. A second collection of this species was made on 12 May 2000.

Seeds were manually extracted. The calyx of *A. graveolens* fruits was removed manually, and the exocarp of *C. brasiliense* fruits was removed by rubbing the fruits over a wire mesh (see photo). After extraction, part of the seeds was used for experiments in the laboratory of CATIE in Costa Rica and another part was sent to DFSC in Denmark, arriving the same week.

Moisture content and germination

For both species, seed moisture content was determined on two to five replicates of 20–25 seeds. Seeds were weighed before and after drying in an oven at 103°C for 17 h. Moisture contents were expressed on fresh weight basis [\pm standard deviation (sd)].

At CATIE, four replicates of 25 seeds were germinated in sand at 30°C with constant light. At DFSC, four replicates of 50 *A. graveolens* seeds were germinated on top of blotter paper at 30°C with 12 h light and 12 h dark, while four replicates of 25 *C. brasiliense* seeds were germinated on vermiculite at 28°C, with 10 h light and 14 h dark. At the end of these tests, seeds that did not germinate were cut to determine whether they were rotten, fresh or empty. Germination percentages and standard deviations of the means were then calculated for each test.

Desiccation trials

To determine the response to desiccation, seeds were mixed with silica gel to dry to different moisture contents and then tested for germination. After determination of initial moisture contents and weights, seeds were monitored for target weights corresponding to specific moisture contents that were calculated following the protocol (DFSC/IPGRI 1999). *A. graveolens* seeds were spread out in one layer in net bags containing silica gel to dry. *C. brasiliense* seeds were mixed with silica gel (2:1) in plastic containers. After desiccation and moisture content determination, samples of seeds were germinated.

Storage trials

At CATIE, samples of desiccated seeds of *A. graveolens* were sealed in plastic and aluminium bags and stored at 15, 5 and -17°C for 2, 4 and 6 months. Samples of dry seeds were stored at 15°C for 12 months. At DFSC, samples of seeds with different moisture contents were sealed in aluminium bags and stored at 15, 5 and -18°C for 2, 4, 6 and 12 months. After each storage period the moisture contents were again determined and seeds were germinated.

For the first trials on *C. brasiliense* at CATIE, seeds with various moisture contents were stored in perforated plastic bags at ambient temperature of $24\text{--}28^{\circ}\text{C}$, at 15 and 5°C for 1, 3 and 6 months. The second storage trials were performed with seeds at two high moisture contents. These were also stored in plastic bags for 6 months.

Results

Desiccation and germination

Astronium graveolens. Initial germination of *A. graveolens* seeds after collection in Costa Rica was $89.0 \pm 3.3\%$, and the moisture content after extraction was $36.6 \pm 1.2\%$. These seeds were dried down to 9.9, 6.4, 3.3 and 1.4% MC at approx. 28°C . Desiccated seeds generally germinated less than the controls, percentages ranging from 55 to 83%. Seeds dried to 1.4% MC, the lowest moisture level germinated 83%, the highest percentage in this trial (Table 1). However, great variations expressed by high standard deviations (3–17%) were observed between the means of replications. On arrival in Denmark, the seeds germinated

65% and had 22% MC (Table 1). The seeds were then dried down to 18.3, 13.0, 8.4, 5.8 and 4.2% at 23°C. Seeds desiccated to 4.2% MC germinated 59%, and there was no significant difference (see standard deviation) between germination percentages in the DFSC trials.

Table 1. Germination capacity (G%±sd) after desiccation of *A. graveolens* seeds to various moisture contents at CATIE and DFSC

| CATIE | | | DFSC | | |
|----------|------------|--------|----------|------------|-----------|
| MC (%) | Drying (h) | G (%) | MC (%) | Drying (h) | G (%) |
| 36.6±1.2 | Initial | 89±3.3 | 21.9±1.0 | Initial | 64.8±11.5 |
| 13.4 | — | 55±9 | 18.3±1.5 | 2 | 69±5 |
| 9.9 | — | 60±17 | 13.0±1.1 | 3.8 | 63±6 |
| 9.4 | — | 76±11 | 8.4±0.5 | 10.6 | 61±12 |
| 6.4 | 7.5 | 72±11 | 5.8±0.5 | 23.7 | 69±13 |
| 3.3 | 32.0 | 68±13 | 4.2±0.3 | 101.6 | 59±10 |
| 1.4 | — | 83±16 | — | — | — |

Calophyllum brasiliense. Initial germination of *C. brasiliense* seeds for the 1999 collection in Costa Rica was 60% and the moisture content after extraction was 43.5% (Table 2). Seeds desiccated to 4.8% MC resulted in a decrease of their germination capacity from 60% to only 4%. This was an indication of great sensitivity to desiccation (Table 2). On arrival in Denmark, seed germination had dropped to 0% with 34% MC. No further action was undertaken on this seed lot.

Table 2. Germination capacity of *C. brasiliense* seeds (collection 1999) after desiccation to various moisture content (%±sd) at CATIE

| Control | | | Dried | | |
|---------------|---------------|---------|------------------|---------------|---------|
| Target MC (%) | Actual MC (%) | G (%) | Drying to MC (h) | Actual MC (%) | G (%) |
| Initial | 43.5±0.9 | 60±22.4 | — | — | — |
| 25 | 40.3±0.6 | 53±15.1 | 72 | 26.3±0.6 | 37±3.8 |
| 20 | 39.2±0.4 | 68±14.2 | 144 | 21.3±0.9 | 22±4.0 |
| 10 | 40.0±1.0 | 64±18.8 | 216 | 10.5±0.5 | 16±14.2 |
| 5 | 41.7±0.8 | 76±8.0 | 360 | 4.8±0.6 | 4±0.0 |

For the second desiccation trial of *C. brasiliensis* (collection 2000), seeds with 34% MC for the second trials had >90% initial germination. There were no significant differences ($P \leq 0.05$) in viability, when seeds were dried to 28% MC, germinating 92% (Table 3). *C. brasiliense* seeds started germinating 3–4 weeks after sowing. Peak germination was observed between 5 and 10 weeks and the total germination period covered 3 months at CATIE and

five months at DFSC (Table 3, Fig. 1). Drying delayed the germination onset, starting 3 weeks later for 28% MC seed compared with the control at 34% MC (Fig. 1).

Table 3. Germination ($G\% \pm sd$) after desiccation of *C. brasiliense* seeds (collection 2000). Mean germination time and germination of controls (*) are also given for the experiments at DFSC

| CATIE | | | DFSC | | |
|------------|----------------|--------------|----------------|---------------------|---------------|
| Drying (h) | MC (%) | G% | MC (%) | Mean G time (weeks) | G% |
| Initial | 56.0 \pm 1.3 | 86 \pm 7.6 | 33.6 \pm 1.1 | 9.09 \pm 0.8 | 95 \pm 3.8* |
| 1.00 | 31.8 \pm 1.2 | 91 \pm 8.9 | 30.9 \pm 1.6 | 8.79 \pm 0.9 | 91 \pm 5.0 |
| 3.67 | 29.8 \pm 0.9 | 91 \pm 3.8 | 34.0 \pm 0.3 | 8.11 \pm 0.7 | 98 \pm 2.3* |
| 5.33 | 28.4 \pm 0.9 | 94 \pm 5.2 | 27.6 \pm 0.7 | 10.57 \pm 0.6 | 92 \pm 3.3 |
| Control | 33.0 \pm 0.9 | 94 \pm 5.2 | 34.4 \pm 0.8 | 7.57 \pm 0.4 | 94 \pm 5.4* |

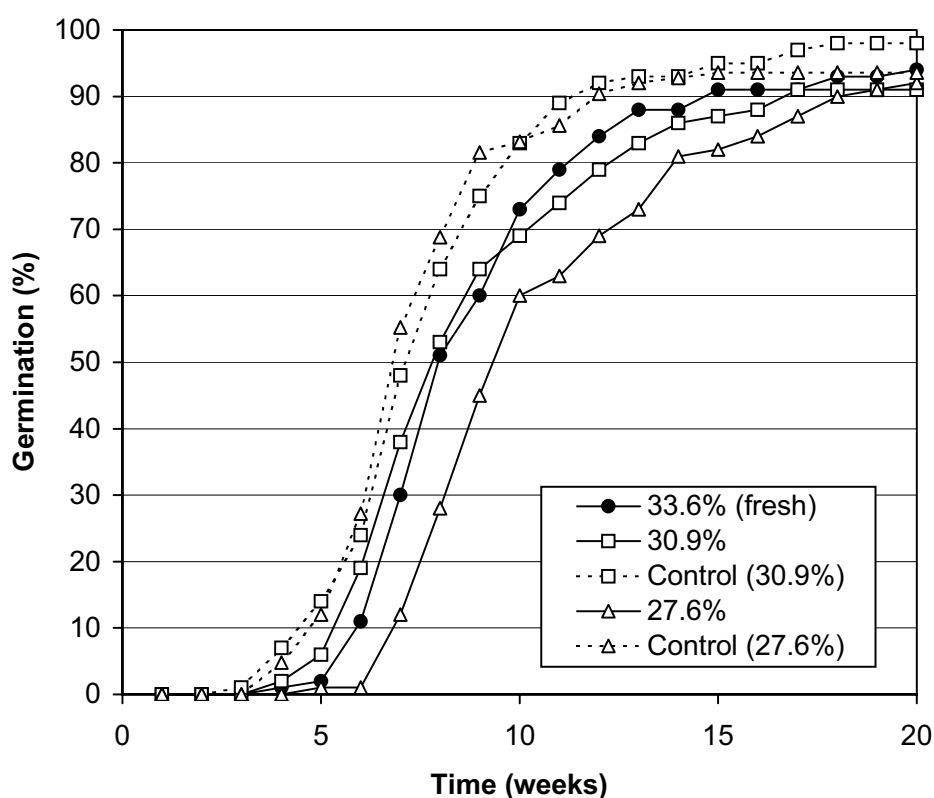


Figure 1. Germination of *C. brasiliense* seeds at different moisture contents at DFSC (the 2000 collection).

Storage

In the CATIE trials, *A. graveolens* seeds at 9.9% MC generally lost viability faster than those with $\leq 6.4\%$ MC (Fig. 2). Seeds with 1.4% MC stored at 15°C for 12 months at CATIE germinated 41% after storage in plastic bags, and 56% after storage in aluminium bags (data not shown). In the DFSC trials, storage results of *A. graveolens* seeds could be separated into two groups. Seeds with $\leq 8.4\%$ MC stored relatively well, with only a slight decrease in their germination capacity, and other seeds with $\geq 13.0\%$ MC that rapidly lost viability, particularly at 5 and -18°C (Fig. 2).

Storage experiments of the first collection of *C. brasiliense* resulted in a better germination of seeds with $>26\%$ MC and at ambient temperature (ca. 26°C), but they were heavily attacked by fungi already after one month. It was clear that *C. brasiliense* seeds were sensitive to desiccation and to low temperatures (Fig. 3).

Discussion

A. graveolens seeds showed great tolerance to desiccation. They could be desiccated to 1.4% MC, maintaining initial viability (Table 1). They also retained similar viability after 6 months storage at CATIE and 12 months at DFSC (Fig. 2). Poor storage results for seeds with the highest MC at low temperatures, indicate MC dependent sensitivity to low temperatures. Recommendations for optimal storage conditions are therefore to dry below 9% MC and store at low temperatures.

C. brasiliense seeds had a very slow germination, covering 5 months (Fig. 1, Table 3) and they were sensitive to drying and low temperatures. Seeds desiccated to 4.8% MC resulted in a decrease of their germination capacity from 60 to only 4% (Table 2). Lines (2001) reported that control seeds germinate faster than dried seeds. This could be due to the difference in initial moisture contents and/or a reduction of vigour in the desiccated seeds. With a safety MC around 30%, the seeds are difficult to store because fungi will attack them. To prevent heavy fungi attack as those observed in trials at both DFSC and CATIE, it would be necessary to improve the disinfecting procedures and/or to store small quantities of seeds in order to reduce the heating by respiration. Transport conditions should be also controlled to avoid receiving dead seed, as was the case for the first collection of *C. brasiliense*, which arrived at DFSC with 0% germination, perhaps due to exposure to low temperatures.

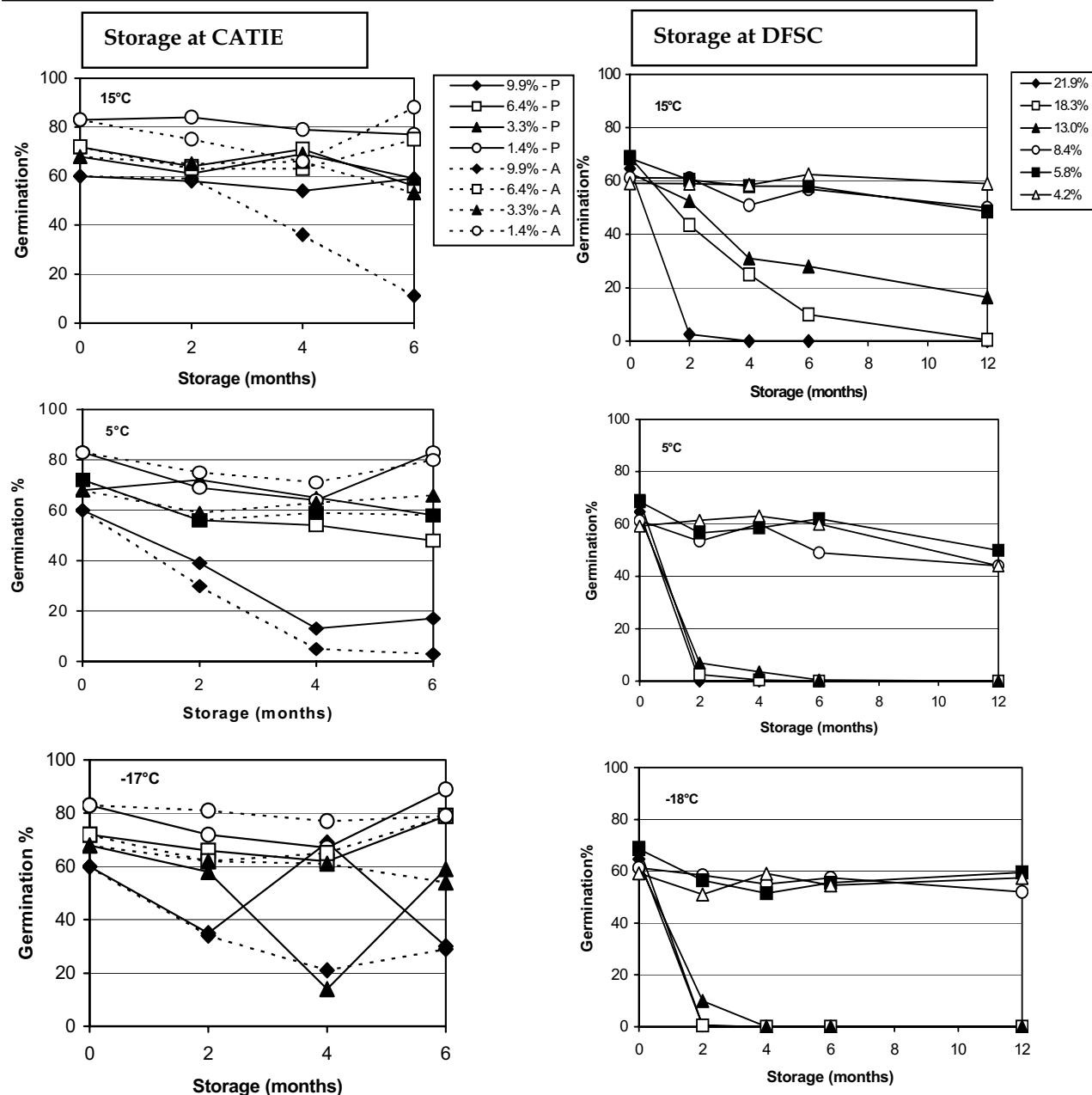


Figure 2. Germination of *A. graveolens* seeds with different moisture contents stored in plastic (P) and aluminium bags (A) for 6 months (CATIE trials). Seeds with different moisture contents were also stored in aluminium bags for 12 months at DFSC (right side graphs).

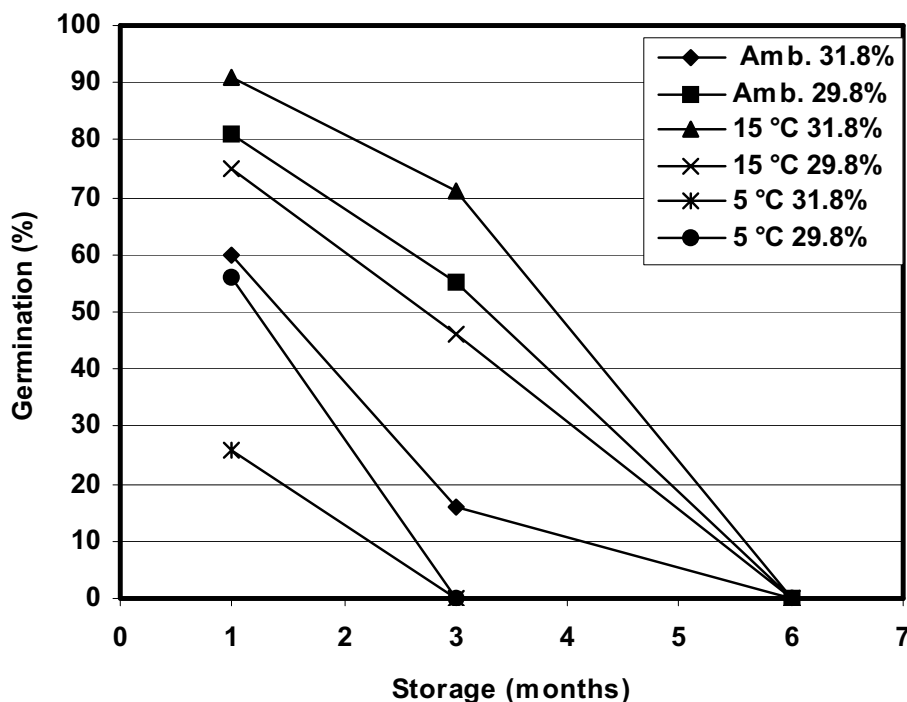


Figure 3. Germination after storage of *C. brasiliense* seeds with 30 and 32% MC at three temperatures for 6 months at CATIE, Costa Rica (the 2000 collection).

Collaboration and future activities

Working within this project was a great experience for CATIE Tree Seed Bank, which gave opportunity to collaborate with seed scientists around the world. Today there are trained people, e.g. a graduate student (see Lines 2001), for screening tree seeds, and the IPGRI/DFSC protocol (1999) is taught as a module to other seed biologists and technicians in Latin America. However, we suggest to include scientific report writing in a future project. The partnership with DFSC has been very enriching. Practice has improved the implementation of the screening protocol (DFSC/IPGRI 1999), which will be necessary to include studies on maturity determination procedure.

For some recalcitrant species, it will be necessary to continue the research activities as a part of conservation strategies. For many endangered native species, it will be necessary to start *ex situ* conservation strategies, establishing seed stand and cryo-conservation research. We propose to create a Recalcitrant tree seed Network that will maintain research communication between scientists and produce scientific publications.

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Drying and storing *Hieronyma alchorneoides* fruits

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Abstract

Hieronyma alchorneoides fruits with an initial moisture content of $52.2 \pm 0.3\%$ and an initial viability of $64 \pm 11.8\%$ were desiccated to 3.3% moisture content without loss of viability. Fruits with four moisture contents between 10.8 and 3.3% were sealed in plastic and aluminium containers and stored at 15, 5 and -18°C . Best results were obtained at 5 and 15°C for fruits stored in sealed plastic bags. After 3 months of storage approximately 50% germination was obtained, however, by 6 months, viability had decreased significantly.

Introduction

In Latin American countries, more attention is now given to indigenous forest species than previously. One species of interest is *Hieronyma alchorneoides* Allemao (pilon) (family Euphorbiaceae) from the very humid tropical forest. It is found up to 900 m above sea level, with an annual rainfall between 2000 and 6000 mm and a mean temperature of 20 to 26°C (Franco 1990). Its distribution extends from Mexico to the Amazonian region and the Antilles. The trees reach up to 45 m tall and 1.2 m of diameter at breast height. The wood is hard (0.63 g/cm^3) and is mainly used for construction.

The fruits are small (ca. 26 500 per kg; 2.5–5.5 mm diameter) oily drupes. The seeds are very small, endospermic and enclosed in a stony endocarp (Flores 1993; Salazar 1997; DFSC 2000). There are few reports about the storage physiology of *H. alchorneoides* fruits. According to Flores (1993) they can be kept for at least 10 days if moisture and temperature are adequate (not specified). COSEFORMA (1998) recommends moisture contents between 5 and 10% at 4°C for storage of the fruits (50% viability at 6 months). The purpose of this study was to define the storage physiology of the fruits and to identify optimal storage conditions.

Materials and methods

Fruit collection and processing

Fruits were collected on Jan 8 and 9 1998 from six trees in PINDECO farm (source No. BL083), Volcan, Puntarenas province (Table 1). At the time of collection, the fruits had changed in colour from green to purple and red, and some had already fallen from the trees. The fruits were removed manually from the clusters and the pulp extracted by manually rubbing the fruits in a sieve.

The fruit of *H. alchorneoides* can be depulped, but usually, including most of the present experiments, this has not been done. Although each fruit contains 3 to 6 seeds, the whole fruit was regarded as one germinative propagule. The fruits were treated with a fungicide (Vitavax) and 1 kg of fruits was sent to Danida Forest Fruit Centre (DFSC), arriving on Jan 20 1998.

Table 1. Site characteristics and quantities of *H. alchorneoides* fruit (source No. BL083)

| Site | Lat. | Long. | Altitude (m asl) | Rain (mm yr ⁻¹) | Temp. (°C) | Collection date | No. trees | Fruit (kg) |
|----------------------------|--------|---------|---------------------|--------------------------------|---------------|--------------------|--------------|---------------|
| Volcan, Buenos Aires | 9°13'N | 83°26'W | 445 | 3630 | 27 | 8/01/98 | 6 | 22 |

Initial tests

In Costa Rica, initial moisture content of fruits including pulp was determined (fresh weight basis). After processing, a sample of 20 depulped fruits was taken and used to measure fruit weight. A fruit containing 3–6 seeds inside was regarded as one entity and measured as only one plant. The initial moisture contents of the fruits were determined on samples of two times 5 g each. Before germination tests, the fruits were rubbed with sandpaper for 30 sec and then soaked in water for 24 h. Four replicates of 25 fruits were incubated for germination in sand at 30°C and in 24 h light.

In Denmark, mean weight and moisture content were determined using five replicates of 50 fruits (IPGRI/DFSC 1996). Cut and TZ tests were carried out on a sample of 100 fruits. Parts of the fruits were dried to estimate their tolerance to desiccation. The germination tests were

carried out using fruits that had been rubbed with sandpaper for 30 sec. Four replicates of 100 rubbed fruits were incubated in vermiculite at 28°C and 12 h light.

Desiccation trials

To determine the minimum moisture content without loss of viability, the fruits were desiccated with silica gel to a range of target moisture contents and tested for germination as before. Controls were kept under similar conditions, but without silica gel, during the desiccation period and a sample sown at the same time as each dried sample.

Storage trials

Fruits with 3.3, 6.8, 9.9 and 11.2% MC were sealed in plastic (P) and aluminium (A) bags (in order to evaluate the effect of packaging material on seed viability), and stored at 15, 5 and -17°C for 3, 6 and 12 months, in Costa Rica. A replicating storage trial was performed on fruits having 4, 7, 10 and 15% MC, and then stored at 15, 5 and -18°C, in Denmark. Germination capacity was tested after 3 and 16 months.

Results

Initial tests

Costa Rica: The initial moisture content of the fruits with pulp was 52.2%, but after manually processing (cleaning), it decreased to $31.6 \pm 0.3\%$ (Table 2). The mean weight of twenty fruits was 7.05 ± 1.96 mg per fruit.

Denmark: The moisture content on arrival at DFSC was 19.2%. Of the 100 fruits that were cut on arrival, 69 appeared fresh and 60 fruits were stained and estimated as viable in the TZ test. Most of the discarded fruits were empty. After 8 months, 9% of the fruits had germinated. The mean weight was 6.8 ± 0.1 mg per fruit.

Desiccation trials

H. alchorneoides seeds could be dried to 3.3–4.5% MC and germinated more than 70% (Table 2). These results from CATIE contradicted what had been found at DFSC, where seeds were received with low initial moisture content (19%). The germination percentage was also low for

experiments carried out at DFSC. However, drying these seeds to about 8% MC improved germination capacity up to 21%. Germination started after four weeks of incubation and ran for a period of 3 months at CATIE and for more than 8 months at DFSC.

Table 2. Germination (G%) of *H. alchorneoides* fruits after desiccation (MC%) under laboratory conditions at CATIE, Costa Rica and at DFSC, Denmark

| CATIE | | DFSC | |
|--------------------|----------|----------|----------|
| MC (%) | G (%) | MC (%) | G (%) |
| 31.6±0.3 (initial) | 64±11.8 | 19.2±1.2 | 8.8±1.0 |
| 16.6±0.4 (control) | 70±10.6 | — | — |
| 15.7±0.3 | 81±18.3 | — | — |
| 12.4±0.3 (control) | 94±7.7 | — | — |
| 11.7±0.2 (control) | 96± 6.0 | 11.5±0.8 | 8.8±4.1 |
| 10.8±0.2 | 65±6.8 | 8.3±1.9 | 10.5±5.2 |
| 8.6±0.3 | 53±10.0 | 7.8±0.9 | 21.5±7.3 |
| 4.5±0.2 | 83± 16.8 | — | — |
| 3.3±0.3 | 70±12.4 | — | — |

Storage trials

Clean fruits with four different moisture contents were stored at three different temperatures for 12 months. Starting with high viability ($\geq 70\%$) after desiccation (Table 2), germination decreased during the first three months of storage at all temperatures (Fig. 1). In the case of fruits stored at 5 and 15°C, after 12 months, there was ca. 20% germination for seeds stored in either plastic (P) or aluminium (A). The viability of fruits stored at -17°C was already less than 20% after 3 months and, in general had fallen further by 12 months storage. In all cases, fruits with lower moisture contents (3–7%) seemed to preserve better compared with those at higher moisture contents (10–11%). There were no significant differences between fruits stored in the plastic bags and those stored in aluminium.

Duplicate storage trials were carried out at DFSC (Table 3). Although starting with low viability at reception, all fruits survived the storage conditions. Germination capacity was maintained for 10–13 months of storage at 5 and 15°C. Fruits with 15% MC did not germinate after storage at -18°C for 7 months. However, the highest germination of 29% was obtained with fruits at 9% MC and stored for 13 months at -18°C.

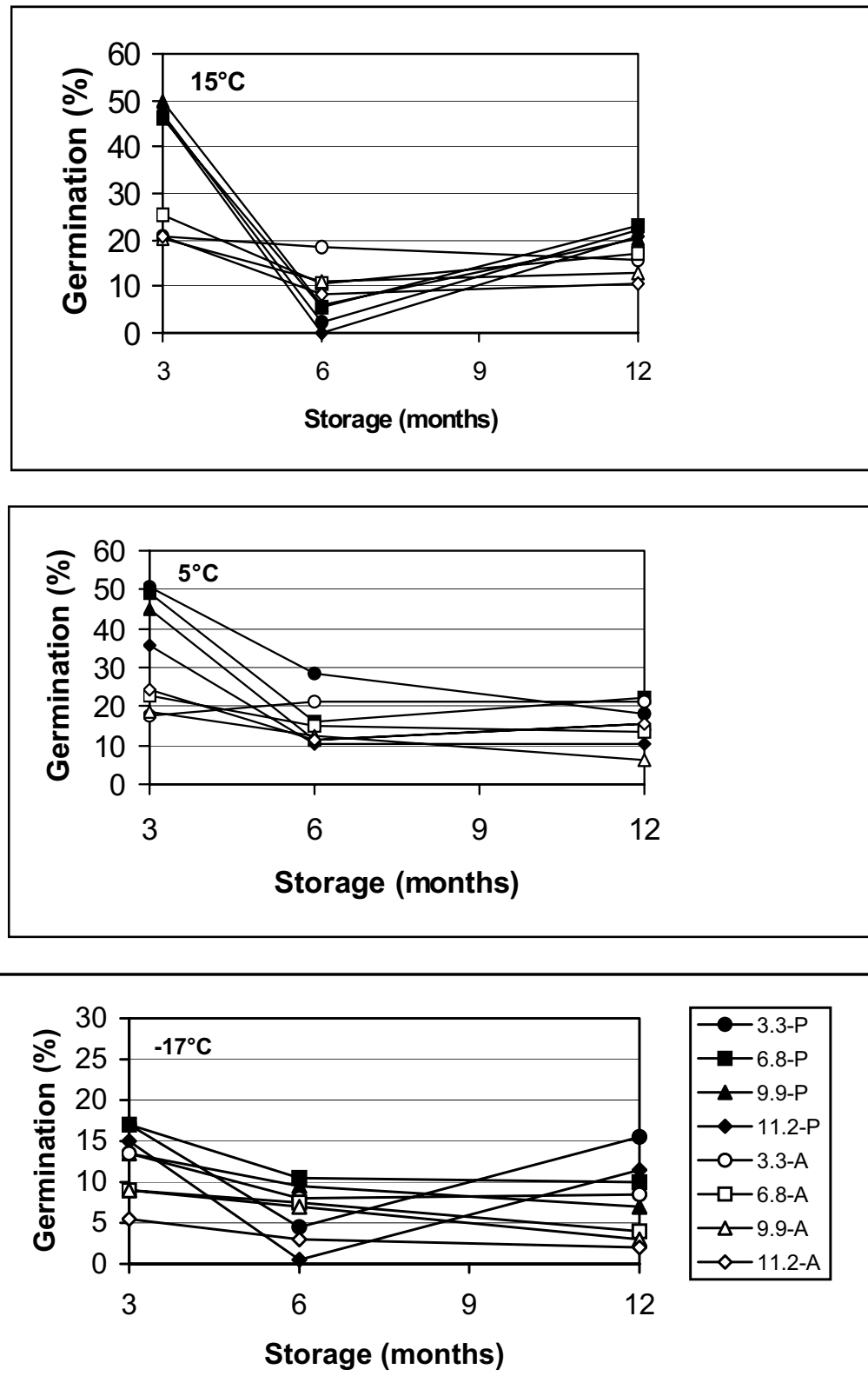


Figure 1. Germination capacity of *H. alchorneoides* fruits with different moisture contents of 3.3 to 11.2% after storage in plastic (P) or aluminium (A) at 15, 5 and -17°C for 12 months.

Table 3. Germination capacity (G%) after desiccation (MC%) and replication of storage trials of *H. alchorneoides* fruits at DFSC

| | Target MC | 15% MC | 10% MC | 7% MC | 4% MC |
|-------|-----------------|-------------|-------------|-------------|-------------|
| 15°C | MC (%) | 15.49±0.42 | 9.62± 0.51 | 7.81± 0.46 | 7.49± 0.62 |
| | G (%) | 7.50±4.80 | 8.75± 3.40 | 10.25± 2.06 | 8.00± 2.00 |
| | Period (months) | 10 | 10 | 10 | 10 |
| 5°C | MC (%) | 16.21± 0.17 | 9.49± 0.55 | 8.63± 0.14 | 7.24± 0.63 |
| | G (%) | 5.50± 2.38 | 6.75± 2.36 | 16.75± 3.20 | 11.00± 6.22 |
| | Period (months) | 10 | 10 | 13 | 10 |
| -18°C | MC (%) | 15.04± 0.44 | 9.18± 0.67 | 8.07± 0.51 | 6.49± 0.39 |
| | G (%) | 0.25± 0.50 | 29.25± 8.50 | 16.00± 7.83 | 13.75± 6.40 |
| | Period (months) | 7 | 13 | 13 | 13 |

Discussion

Desiccation trial

The viability of *Hioronyma alchorneoides* fruits dried to moisture contents as low as 3.3% remained high ($\geq 70\%$). This is in accordance with previous experiments where *H. alchorneoides* seeds could survive drying to 7.5% MC (Trivino *et al.* 1990). However, replicating experiments at DFSC showed very low percentages (9 to 22%) of germination, although the TZ test performed on the fruits on arrival indicated 60% viability of the seed lot. Sub-optimal conditions at DFSC might have been the reason for the very poor germination. Because they could be dried to very low moisture content and still maintained high viability, it can be concluded that the fruits of *H. alchorneoides* are tolerant to desiccation.

Even with pretreated fruits, germination first started after four weeks and was spread overlong periods. The germination window varied from 3 months at CATIE to more than 8 months at DFSC, possibility due to some dormancy induced during the transit to Denmark. COSEFORMA (1998) presented similar results with germination starting 17 days with pretreated fruits and 24 days without pretreatment. However, in a separate trial, when fruits from this same seed lot were sown in mixed sand and loam (1:1) in a greenhouse, they completed germination within only 30 to 40 days. Because these fruits were covered with a very thin mix of sand and loam, we suspect light sensitivity may play a role in the germination process of this species.

Storage trial

After 3 months storage, the best results of up to 50% germination were obtained for fruits stored in plastic bags at 5 and 15°C (Fig. 1). However, their viability decreased significantly after 6 and 12 months. The poor storage results at -17°C at CATIE (<20%) seemed to indicate sensitivity of *H. alchorneoides* seeds to low temperatures. This was not supported by the results obtained from DFSC (Table 3), where the germination of fruits was at the highest after 13 months storage at -18°C.

A preliminary conclusion of these trials is that the fruits are fully desiccation tolerant, but short-lived. However, more investigations are needed to establish optimal conditions for germination and storage.

Acknowledgements

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Drying and storage of *Vochysia ferruginea* seeds

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Abstract

Desiccation tolerance and the effects of moisture content and storage temperature on seed longevity were investigated on *Vochysia ferruginea* from two sources of Costa Rica. The initial moisture content of seeds at harvest was 39%, and they had high initial viability, greater than 90% germination. Seeds tolerated drying to 6.4% MC, maintaining their initial viability. Fungal growth was important during the germination tests, which greatly contributed to the reduction of the germination percentage. Viability declined faster for seeds stored at low temperatures and no seeds survived storage at -17°C. Seeds with 9.6 and 12.3% MC germinated 51% after 6 months of storage at 15°C. However, none of these seeds survived after 12 months. It was recommended to store seeds of *V. ferruginea* with 8–10% MC at 15°C for a few months.

Introduction

Vochysia ferruginea Mart. tree from the Vochysiaceae family grows up to 35 m and 80 cm diameter at breast height. It has a straight trunk, free of branches to half its height. It grows in low areas, in less fertile, acidic but well-drained soils, usually with a slope. The tree easily colonizes abandoned lands. The wood of *V. ferruginea* is of great interest to loggers because of its high timber value. It is light with a specific weight of 0.38 g cm⁻³, dries easily and is easy to work. It is used in construction, furniture making and craftwork (Salazar 1997).

When planted, the initial growth of seedlings is relatively slow, but accelerates in the second or third year. In the North Huetar region of Costa Rica, the trees can grow 3.4 m in 2 years after planting (Rodriguez 1997). Flowering usually occurs from Mar to Apr, and the trilocular fruits ripen from June to Sept, turning from green to brown. Mature fruits should be collected directly from the trees when they change

colour and before they open (Salazar 1997). *V. ferruginea* tree has a regular peak production of fruits every 2 years.

There is little and contradictory information on the physiology of *V. ferruginea* seeds. According to Flores (1993), seeds must not be dried and should be kept at 24–26°C to maintain viability. However, seeds from different sources have been found to tolerate desiccation, but stored best nondried at 15°C, resulting in 64% germination after 3 months, and 23% germination after 4 months (Müller 1997). The purpose of this study was to establish optimal storage conditions with regard to seed moisture content and storage temperature.

Materials and methods

Seed maturity and collection

In order to identify the best time for collection, fruits were harvested every 2 weeks and seed samples were examined to determine their level of maturity that was also associated with recorded fruit colour. It was then determined that mature fruits should be collected when their colour turned from light green to dark green with marked divisions between the locules. The fruits matured irregularly throughout the tree crowns.

Fruits were collected from 10 trees of Volcán, Buenos Aires, Puntarenas (Bl082) in Sept 1997 and from three trees of Cajón de San Pedro, San Isidro Puntarenas in March 1998. These sites are located in 9° N latitude and 83° W longitude, at more than 500 m above sea level, where the mean temperature was 23°C and the annual rainfall attains nearly 3000 mm. Fruits in sacks were transported to the Tree Seed Bank at CATIE 1 day after collection, and were kept in the shade for 2 days before the seeds were manually extracted.

For replicating experiments, half of the seeds were sent by courier to both DFSC and the Agricultural University in Copenhagen, arriving 3 to 9 days after.

Initial tests

Seeds collected from Volcán were used in the desiccation trials and the other seeds from San Pedro were used in the storage trials. Fruit and seed characteristics were determined using 100 individuals, mainly from the seed lot of 1997 collection. The fruits and seeds were weighed and measured, and the mean values were calculated.

Desiccation and storage trials

Seeds were mixed with silica gel in plastic bags and the bags placed at ambient conditions (24 to 28°C and 70 to 90% RH). Samples of seeds were used to determine the moisture content of the seeds, gravimetrically by weighing them before and after oven drying at 103°C for 17 h. The moisture content was then determined as a percentage of the seed fresh weight.

Seeds were dried to five moisture contents between 21.1 and 6.4% and were tested for germination capacity. Desiccated seeds with four moisture contents between 12 and 8% were stored at 15, 5 and at -17°C for 3, 6 and 12 months. The seeds were treated with NaOCl before the storage trials. For germination, all seeds were sown in sterilized sand and put to germinate at 30°C in constant light.

Results

Initial tests

The seeds sent to the Agricultural University of Copenhagen had very low viability on arrival, thus there were not enough seeds for the replication of all experiments. Only, the results of tests carried out at the Tree Seed Bank at CATIE are presented. Fruit and seed characteristics were determined using 100 individuals. The seed composed about 5% of the fruit (Table 1). There were ca. 30 000 seeds per kg and more than two seeds on average per fruit.

Table 1. Initial characteristics and quantities of *V. ferruginea* fruits and seeds from Costa Rica. The mean values of weights and dimensions are for 100 individuals

| Year | Fruit | | | | | MC (%) |
|------|--------------|------------|-------------|------------|--------------|----------|
| | Harvest (kg) | Weight (g) | Length (cm) | Width (cm) | Seeds /fruit | |
| 1997 | 145.2 | 1.46±0.20 | 2.99±0.21 | 1.17±0.08 | 2.64±0.59 | |
| 1998 | 27.0 | — | — | — | — | |
| | Seed | | | | | MC (%) |
| | Harvest (kg) | Weight (g) | Length (cm) | Width (cm) | Seeds /kg | |
| 1997 | 4.5 | 0.08±0.02 | 2.67±0.32 | 0.55±0.25 | 30 000 | 39.0±0.3 |
| 1998 | 0.50 | — | — | — | — | — |

Desiccation and storage trials

V. ferruginea seeds had 39% initial moisture content (Table 1). All experiments started with seeds at 21.1% MC, and drying these seeds to the first target moisture content took approximately 6 h in silica gel to reach 12.1% MC, whereas the driest moisture content of 6.4% was obtained after 12 h. Seeds with 21.1% MC initially germinated to 94%. However, desiccating *V. ferruginea* seeds to 6.4% MC did not result in any loss of viability; seeds retained 93% germination (Table 2).

Table 2. Germination (G%) of *V. ferruginea* seeds from BI082 (1997) after desiccation to different moisture contents (MC%)

| Control | | | Desiccated | |
|---------------|--------|-------|------------|-------|
| Target MC (%) | MC (%) | G (%) | MC (%) | G (%) |
| Initial | 21.1 | 94 | 21.1 | 94 |
| 20 | 17.8 | 92 | 12.1 | 98 |
| 15 | 16.6 | 85 | 11.7 | 93 |
| 10 | 17.3 | 94 | 8.9 | 96 |
| 5 | 14.7 | 89 | 6.4 | 93 |

Table 3 shows the results of the storage trials after 3 and 6 months. There was a great increase from 31% after 3 months to 51% germination after 6 months storage at 15°C for seeds with 12% MC. This maybe due to fungal contamination during the germination tests. At drier moisture contents (<12%), viability of seeds was relatively constant over 6 months storage at 15 and 5°C. No seeds germinated after 12 months of storage. All seeds did not withstand storage at -17°C, losing viability already within 3 months. The analyses of variances showed that there were significant differences ($P < 0.001$) between seeds with higher moisture content (11–12%) and those with lower moisture content (7.9%), after 6 months at 15 and 5°C.

Discussion

Monitoring fruit/seed development of *V. ferruginea* allowed to identify peak maturity and to harvest high quality seeds germinating 100% for this study. The seeds were small, c. 30 000 seeds per kg, and composed about 5% of the fruit mass, which was also reflected on the huge quantity of fruits harvested (Table 1). The transport conditions should therefore be revised to avoid losses of such valuable materials, by shortening its duration and/or controlling travel conditions in such a way that seeds can be received with less deterioration. Replication of present data would have helped confirm or infirm some of the findings.

Table 3. Germination of *V. ferruginea* seeds from Cajón, San Pedro (1998) after desiccation and storage. * = different letters indicate significant differences (at P=0.1% level, Least Squares Means) per storage period

| MC (%) | | Germination (%) | Storage (°C) | Germination±sd (%) after storage | | |
|--------|--------|-----------------|--------------|----------------------------------|---------------------|-----------|
| Target | Actual | | | 3 months | 6 months | 12 months |
| 12 | 12.3 | 93 | 15 | 31±8 ^{2,3*} | 51±6 ¹ | 0 |
| | | | 5 | 30±5 ³ | 26±4 ^{3,4} | 0 |
| | | | -17 | 0 | 0 | 0 |
| 9 | 11.2 | 98 | 15 | 43±7 ² | 42±7 ^{1,2} | 0 |
| | | | 5 | 30±7 ³ | 18±7 ⁴ | 0 |
| | | | -17 | 0 | 0 | 0 |
| 6 | 9.6 | 99 | 15 | 60±5 ¹ | 51±8 ¹ | 0 |
| | | | 5 | 38±2 ² | 32±5 ^{2,3} | 0 |
| | | | -17 | 0 | 0 | 0 |
| 3 | 7.9 | 100 | 15 | 59±9 ¹ | 35±8 ^{2,3} | 0 |
| | | | 5 | 42±4 ² | 35±8 ^{2,3} | 0 |
| | | | -17 | 0 | 0 | 0 |

V. ferruginea seeds tolerated desiccation to 6.4% MC, maintaining their initial viability of ca. 93% germination. Thus, these seeds were fully desiccation tolerant and would be expected to store for long periods of time at low temperatures. However, the storage results showed that all seeds did not withstand storage at -17°C, losing viability already within 3 months, and no seeds germinated after 12 months at 5 and 15°C (Table 3). The handling of seeds after storage at -17°C might have not been suitable to allow their survival, particularly at low MCs (see Table 3). After 3 months of storage, the viability dropped markedly, particularly for seeds with 11–12% MC, which was partly due to fungal contamination during the germination test. Dry seeds to 7.9 and 9.6% MC maintained ca. 60% germination, the highest percentage after storage at 15°C for 3 months, but not during the 12 months storage. Although, seeds looked fresh after the 12 months storage, they did not germinate, but were completely covered by opportunistic fungi. These seeds have a similar behaviour to *V. guatemalensis* (Salazar *et al.* 1996).

The mean germination after 6 months was 45% for all the 15°C treatments and 28% for all the 5°C treatments, and these two results were significantly (P<0.01) different (Table 3). Thus, *V. ferruginea* seeds, stored better at 15°C for at least 6 months. Other studies did not provide good survival of seeds at 7 and 12% MC, and therefore suggested also that nondried *V. ferruginea* seeds be stored at 15°C

(Müller 1997). However, due to a great variation between different seed sources (Müller 1997), it is difficult to recommend a single optimal moisture content for this species. For these studied sources, seeds can be dried to 8–10% MC for the short-term storage at 5 or 15°C.

Conclusions

Although, *V. ferruginea* seeds were tolerant to desiccation, they were also relatively short lived and sensitive to low temperatures (below 0°C). It is recommended that seeds are stored at 8–12% MC and 15°C for a few months. More investigations are needed on the effect of moisture content and storage temperature, as well as germination conditions controlling fungi.

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Seed storage behaviour of *Vochysia guatemalensis*

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Abstract

Desiccation tolerance and optimal conditions of moisture content and temperature during storage were investigated on *Vochysia guatemalensis* seeds collected from two sources in Costa Rica in 1996 and 1997. Seeds with an initial 43% moisture content germinated 100%. They tolerated drying to 5% MC, maintaining more than 90% germination. Seeds desiccated to 10.4% MC stored at 5°C and 15°C, retained 68 and 58% germination after 6 months. However, a maximum of 29% of seeds survived storage at 15°C after 12 months. Although the seeds were desiccation tolerant, storability still posed problems, as most of the seeds lost viability within 1 year storage. Furthermore, even very dry seeds could not be stored at -17°C. It is recommended that the seeds are stored at ca. 10% MC and at 15°C and that the relationship between moisture content and temperature is investigated further.

Introduction

Vochysia guatemalensis Donn. Sm. is a tall tree with yellowish, soft wood that grows in the humid tropical forest, up to 800 m above sea level along the Atlantic Coast from Mexico to Panama. The trees grow up to 40 m in height and 180-cm diameter at breast height. The trunk is straight, cylindrical and free of branches. The species grows well on different soils like flooded to loamy sand, loamy clay and compact soils. It forms pure stands in abandoned pasture and agricultural lands. The grain is straight and the wood, which is easy to work, is used for construction and furniture (Salazar 1997). Good establishment of experimental plantings has made the species highly attractive in forest plantations. However, the lack of knowledge is still a problem for better handling of the seeds.

Flowering begins when the trees are six to eight years old and usually occurs between March and June, and the fruits mature from August to October. The winged seeds are flat and pubescent and should be collected from the tree before the trilocular capsule fruit opens (Salazar 1997). According to Flores (1993), fresh seeds with 32% MC stored at 5°C, rapidly lose their viability. At 10 and 25% MC, they germinated 70 and 75%, respectively, after six months storage. Müller (1997) found that drying seeds to 11% MC (45% relative humidity) and storing them at 20°C were the best conditions, as approximately 75% germinated after four months and 30% after six months of storage. For seeds dried to 5% MC and stored at 4 and 15°C, their viability was maintained above 50% germination after three months. Only 15% of seeds with 5% MC germinated after six months at -15°C. The present investigations aimed to determine the best storage conditions for *V. guatemalensis* seeds and thereby prolonging the storage period.

Materials and methods

Seed collection and extraction

Fruits of *V. guatemalensis* were collected from 10 to 14 trees of natural stands and plantations in La Argentina de Pocora, Limón (BL063) and San Rafael, Pérez Zeledón (BL077) on the 27 of June 1996 and the 13 of Aug 1997 (see Table 1). Before collection, the fruits were monitored every two weeks and it was determined that mature fruits should be collected when their colour turned from light green to dark green with marked divisions of the locules.

Fruits were transported to the Tree Seed Bank at CATIE in sacks the day after collection. The ambient temperature varied between 24 and 29°C and the relative humidity between 60 and 80%. The fruits were dried for two days in the shade and the seeds were extracted manually (Table 1). Extracted seeds, from both years, 1996 and 1997, were split into two, to be used for replicating experiments at CATIE and at Danida Forest Seed Centre (DFSC).

Table 1. Site characteristics and quantities of *V. guatemalensis* seed lots collected in 1996 and 1997

| Site | Latitude (N) | Longitude (E) | Altitude (m) | Rainfall (mm) | Fruits (kg) | Seeds (kg) | Ratio (S/F) |
|------------------------------|--------------|---------------|--------------|---------------|-------------|------------|-------------|
| Limón (BL063) – 1996 | 09°18' | 83°31' | 740 | 2934 | 192.7 | 7.0 | 0.036 |
| Pérez Zeledón (BL077) – 1997 | 10°07' | 83°38' | 250 | 3722 | 66.7 | 1.5 | 0.022 |

Desiccation and germination trials

Costa Rica

Seeds were desiccated in silica gel at 27°C (ambient temperature) to reach seven target moisture contents of 30, 20, 16, 13, 10, 8 and 5%. After desiccation, replicates of seeds were used to determine moisture content gravimetrically by weighing them before and after oven drying at 103°C for 17 h. Moisture content was then calculated as a percentage on a fresh weight basis.

Four replicates of 50 seeds, for each treatment, were germinated in sterilized sand (treated with 5% Formalin), in a germination cabinet at 30°C, 80% RH and constant light.

Denmark

The desiccation trials were replicated at DFSC using the same methods except that the seeds were germinated in vermiculite, instead of sand. Temperature and light were similar to the conditions at CATIE.

Storage trials

Costa Rica: Seeds with 3, 6, 9 and 12% MC were packed in transparent plastic bags and stored at -17°C, 5°C and 15°C where after, the seeds were germinated (same conditions as above). Seeds sent to DFSC were dead on arrival.

Results

Initial tests

Initial characteristics of fruits and seeds were similar for 1996 and 1997 collections. Fruits and seeds from Limón–BL063 were bigger (9 and

0.45 g, on average) than those from Pérez Zeledón—BL077 (7.8 and 0.39 g, on average), resulting in 5800 seeds per kg for the former source compared to 6139 seeds per kg for the latter source. Both seed lots had high initial MCs of 42–45% (Table 2).

Table 2. Initial characteristics of *V. guatemalensis* fruits and seeds

| Source | Fruit | | | | MC (%) |
|--------------|------------|-------------|------------|-------------|--------|
| | Weight (g) | Length (cm) | Width (cm) | Seeds/fruit | |
| BL063 (1996) | 9.02 | 4.70 | 1.02 | 2.50 | |
| BL077 (1997) | 7.80 | 5.76 | 1.96 | 2.76 | |
| | Seeds | | | Seeds/kg | MC (%) |
| | Weight (g) | Length (cm) | Width (cm) | | |
| BL063 (1996) | 0.45 | 5.67 | 1.95 | 5800 | 45.3 |
| BL077 (1997) | 0.39 | — | — | 6139 | 42.3 |

Desiccation and germination trials

Seed samples were dried at CATIE to eight moisture contents and tested for germination capacity. Seeds began to germinate 8–9 days after sowing, and germination took another 4 to 11 days. Seeds with an initial 43% MC and 100% germination, tolerated drying to 5% MC with only a slight decrease in germination (Table 3). Similar results were obtained at DFSC, where germination percentage remained the same for non dried and dried seeds, although the MC of these seeds was reduced to 27% at arrival.

The energy of germination was calculated as the percentage of germinated seeds 3 days from the onset of the test to the total number of seeds that germinated. Seeds with <15% MC germinated faster (high energy) than seeds with higher moisture contents (low energy) (Table 3). Experiments at DFSC showed similar onset of germination after sowing, but germination finished after 15–19 days.

Analysis of variance (ANOVA) confirmed that there were no significant differences between the treatments at both laboratories.

Storage trials

Seeds were desiccated to 12.1, 10.4, 6.7 and 2.2% MC, resulting in, respectively, 93, 95, 90 and 84% germination, and were stored at -17, 5 and 15°C for 3, 6 and 12 months. No seeds germinated after storage at -17°C (Fig. 1). Most seed samples stored at 5 and 15°C already showed a significant decrease in viability after three months storage (Fig. 1A). Germination was between 48 to 76% for seeds with <12% MC at 5°C after 3 and 6 months. At 12% MC, seeds germinated ca. 30% after 3 months, but 48% after 6 months (Fig. 1A and 1B). For seeds with >3% MC, germination percentages after storage at 15°C for 3 and 6 months were between 41 to 86 (Fig. 1A and 1B). At 2.2% MC seeds germinated respectively 13, 20 and 20% after storage at 15°C for 3, 6 and 12 months. After 12 months of storage at 15°C, between 18 and 29% germination was obtained, the best result being for seeds with 10.4% MC (Fig. 1C).

Table 3. Germination (G% and energy) responses of *V. guatemalensis* seeds to desiccation

| Target MC (%) | CATIE | | | DFSC | |
|---------------|--------|-------|--------------|--------|-------|
| | MC (%) | G (%) | G energy (%) | MC (%) | G (%) |
| Control | 43.0 | 100 | 40 | 27.9 | 96 |
| 30 | 33.3 | 100 | 43 | — | — |
| 20 | 17.8 | 100 | 52 | 21.5 | 94 |
| 16 | 14.9 | 100 | 80 | 17.2 | 92 |
| 13 | 12.0 | 100 | 82 | 13.7 | 96 |
| 10 | 9.1 | 100 | 94 | 10.2 | 92 |
| 8 | 8.0 | 99 | 99 | 9.9 | 93 |
| 5 | 5.0 | 97 | 59 | 5.9 | 96 |

Discussion

Fresh seeds of *V. guatemalensis* were harvested at peak maturity, which was determined after preliminary observations and tests. The seeds had high initial 43% moisture content and high viability of 100% germination. They, however, tolerated desiccation down to 5% MC, maintaining initial viability. This also confirmed the results of Salazar *et al.* (1996). Despite the small delay of about a week in receiving seeds in Denmark, these results were reproduced at DFSC (see Table 3), and

there were no significant differences between the treatments at both laboratories. The energy of germination calculated at 3 days of the germination onset, allowed us to demonstrate that drying increased the rate of germination (Table 3), seeds with <15% MC germinating faster than those with higher moisture contents.

To estimate their longevity, desiccated seeds were stored at different temperatures for 12 months at CATIE. Most seed samples stored at 5 and 15°C showed a significant decrease in viability after three and six months storage, and a maximum of 29 ± 3 and $24 \pm 5\%$ germination was obtained for seeds with 10.4 and 6.7% MC stored at 15°C (Fig. 1). Seeds with 12% MC may have been chill-injured at 5°C, reducing their germination to <50%. By contrast seeds dried to 2.2% MC had reduced but constant germination of 13–20% over 12 months at 15°C. This indicated that drying did not improve storage of *V. guatemalensis* seeds at 15°C. However, the combination of 10.4% MC and 15°C resulted in the highest germination at all three storage durations. Furthermore, Flores (1993) and Müller (1997) recommended storage of these seeds at more than *ca.* 10% MC and at 15°C.

One might predict that seeds tolerating desiccation to 5% MC, would also be storable at low temperature, as usually found in many desiccation tolerant seeds. However, no seeds germinated after storage at -17°C (Fig. 1), this is in accordance with another study of *V. guatemalensis* from several seed sources, where the seeds also showed poor storage survival at -15°C (Müller 1997). As for neem, another tropical species, dry seeds must be handled with caution when rehydrating them for germination, to avoid imbibitional damage (Sacandé *et al.* 1998). In addition, seeds taken from sub-zero temperatures should be 'acclimated' at room temperature for at least 24 h before germination tests. This fluidises cell membranes and avoids cell death by rigid membrane rupture (Hoekstra *et al.* 1999). Lack of such precautions may have affected the germination capacity of these seeds.

Sensitivity to low temperatures may explain why the seeds arrived dead at DFSC in 1997, as they may have been exposed to freezing temperatures at the plane. Hence, taken together, it was still unclear from the present study, what the optimal storage conditions of *V. guatemalensis* seeds are. The relationship between moisture content and storage temperature should be investigated more thoroughly, as well as types of seed storage containers.

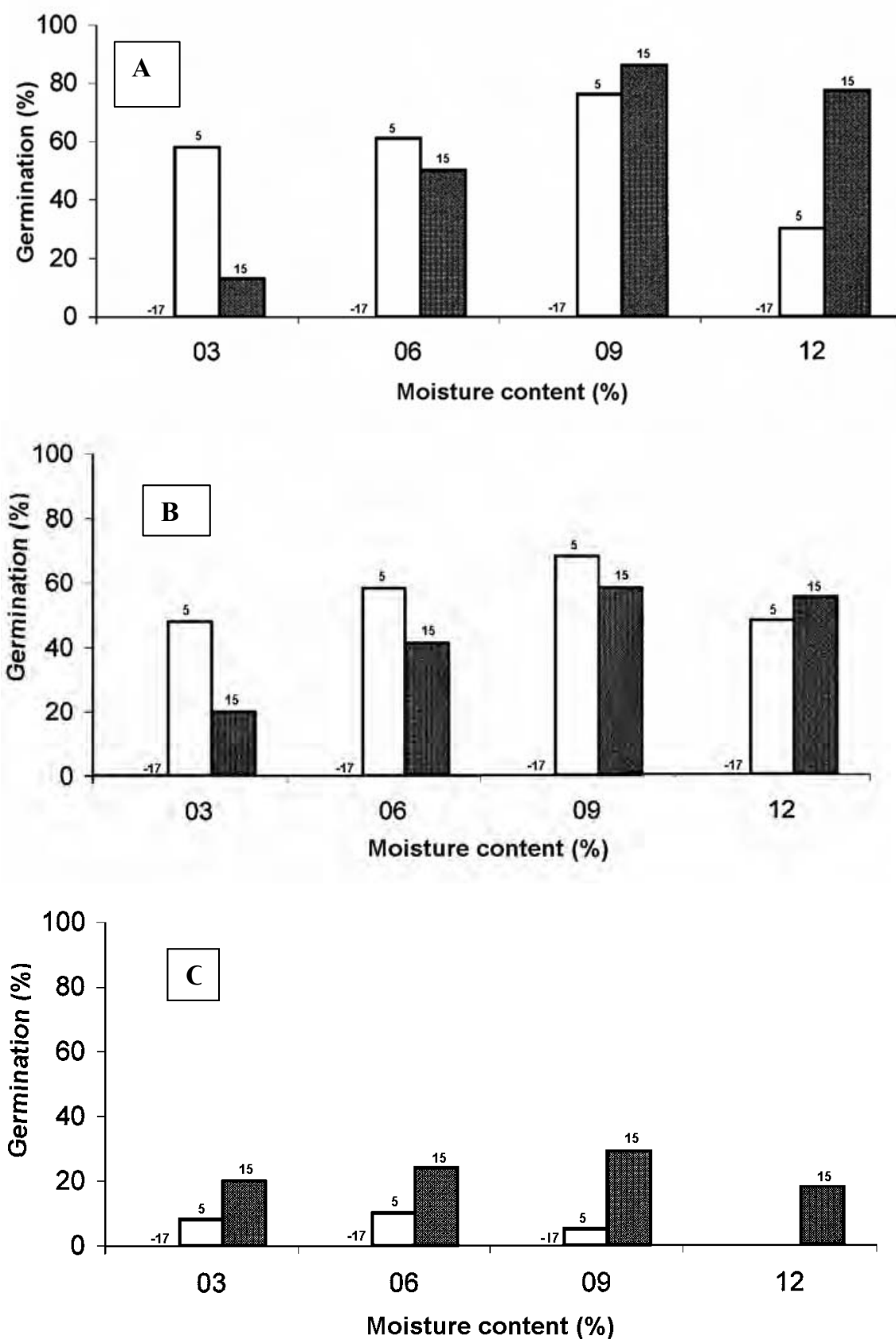


Figure 1. Viability of *V. guatemalensis* seeds with 2.2, 6.7, 10.4 and 12.1% MC, after storage at -17 , 5 and 15°C . Seeds were stored for 3 (A), 6 (B) and 12 (C) months at CATIE.

Conclusions

Although the seeds of *V. guatemalensis* were tolerant to desiccation to 5% MC and maintained a high initial viability, storability still posed problems, as seeds could not be stored for more than six months, losing greatly their germination capacity. The seeds do not tolerate -17°C. On basis of the results and literature it is recommended to store the seeds with ca. 10% MC and at 15°C.

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