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**To cite this article:** Antonia Nyamukuru, John R. S. Tabuti & Philip R. Aduma (2014) Propagation and seedling establishment of selected multipurpose woody species of Uganda, International Journal of Biodiversity Science, Ecosystem Services & Management, 10:4, 270-274, DOI: [10.1080/21513732.2014.953584](https://doi.org/10.1080/21513732.2014.953584)

**To link to this article:** <http://dx.doi.org/10.1080/21513732.2014.953584>



Published online: 02 Sep 2014.



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## Propagation and seedling establishment of selected multipurpose woody species of Uganda

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Cultivation of important woody species with the goal of conserving them is hampered by little, or no, information on how such species can be propagated or their seedlings established. We investigated propagation and seedling establishment of 14 important multipurpose species much valued for traditional medicine in Uganda. The experiments included sexual and vegetative propagation, as well as a seedling growth experiment. The multipurpose species *Psidium guajava*, *Markamia lutea*, *Maesopsis eminii* and *Spathodea campanulata* propagated easily from seed, and *Ficus natalensis* from cuttings. The medicinal species *Zanthoxylum lepreurii*, *Warburgia ugandensis* and *Ficus saussureana* did not germinate at all. *Toddalia asiatica* and *Canarium schweinfurthii* had high dormancy and low synchrony of seed emergence. *Markamia lutea* seedlings established readily with addition of fertilizer (NPK) and moderate watering (three times a week). We conclude that whereas the multipurpose use species appear to propagate easily, the medicinal plants are difficult to propagate sexually or asexually.

**Keywords:** medicinal species; germination; seedling performance; re-forestation; agroforestry

### 1. Introduction

Globally, many households and communities depend on trees for the provision of goods and services that include fuelwood, edible fruits or herbal medicines. Trees also provide a more global service by facilitating carbon sequestration with the potential for climate change mitigation (Albrecht & Kandji 2003). Multiple use tree species that provide a variety of products are generally preferred over single use species as they satisfy different household needs and buffer household vulnerabilities to changing market conditions (Scherr 1995). Many people rely on traditional medicines (TM), especially those in remote and rural areas (Hamilton 2004). It is estimated that 70–80% of the people worldwide depend on herbal medicines, which also includes those who derive their income from gathering and processing of herbal medicines. Much of the material used in TM is sourced from tree species, which for the most part are wild rather than cultivated forms. It is believed that many of these tree species especially those sourced from the wild are threatened by habitat conversion or overexploitation (FAO 2009); because of this and the value attached to the tree species used for TM, conservation through domestication and planting becomes a necessity.

Lack of knowledge, especially in propagation techniques for woody species, inhibits farmers in Africa, including Uganda, from successfully growing these trees and shrubs (Schreckenberget al. 2002; Akinnifesi et al. 2008). According to Maghembe and Prins (1994), there is inadequate research into planting and regeneration of indigenous woody species in Africa; and most of the work to date has been skewed towards exotic tree

species. Consequently, there is an urgent need to research and improve both new and existing methods for propagation and regeneration of indigenous tree species (Wightman 1999).

Plants are propagated by sexual or vegetative techniques; sexual propagation by seed being important as it maintains genetic diversity and is also cheaper than asexual techniques (Hartmann et al. 2011). However, dependence on seed may not be the best solution from the farmer's point of view as seed progeny can be highly variable and also have long emergence periods so there is significant time delay before being able to harvest useful products. Vegetative propagation, on the other hand, may lead to low genetic variability but yield offspring that are highly precocious and enable farmers to access products within a short timescale (Akinnifesi et al. 2008; Hartmann et al. 2011; Asaah et al. 2012).

This study was conducted to determine the propagation and seedling establishment of selected, highly valued medicinal and multipurpose woody species. The results could contribute to the conservation of some important woody species.

### 2. Method

Two propagation experiments and one seedling growth experiment were carried out at a nursery garden in Masoli Village, Wakiso District, 12 km from Kampala, the capital city of Uganda. The study comprised the following experiments: sexual propagation using seeds, vegetative propagation using leafy stem cuttings and seedling growth using results from seed propagation. The 14

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Table 1. Multipurpose medicinal species included in the experiments, (a) seed germplasm and (b) leafy stem cuttings germplasm. The dates of collection and areas of origin of the germplasm are shown.

Species	Local name	Date of collection	Provenance
<b>(a) Seeds</b>			
<i>Canarium schweinfurthii</i> Engl.	Mpafu	September 2011	Mpigi
<i>Cordia africana</i> Lam.	Mukebu	August 2011	Kapchorwa*
<i>Entada abyssinica</i> Steud. ex A. Rich.	Mwolola	October 2011	Mpigi
<i>Eucalyptus globulus</i> Labill.	Kalintusi	October 2006	Kabale*
<i>Maesopsis eminii</i> Engl.	Musizi	October 2011	Mpigi
<i>Maesopsis eminii</i> Engl.	Musizi	July 2011	Mayuge*
<i>Markhamia lutea</i> (Benth.) K. Schum.	Musambya	January 2011	Luwero*
<i>Milicia excelsa</i> (Welw.) C.Berg	Muvule	June 2010	Palisa*
<i>Psidium guajava</i> L.	Mapera	October 2011	Mpigi
<i>Spathodea campanulata</i> P. Beauv.	Kifabakazi	November 2011	Mpigi
<i>Toddalia asiatica</i> (L.) Lam.	Kawule	October 2011	Mpigi
<i>Zanthoxylum leprieurii</i> Guill. & Perr.	Munyenye	October 2011	Mpigi
<b>(b) Cuttings</b>			
<i>Ficus natalensis</i> Hochst.	Mutuba	November 2011	Wakiso
<i>Ficus saussureana</i> DC.	Muwo	December 2011	Mpigi
<i>Warburgia ugandensis</i> Sprague	Abasi	December 2011	Mpigi
<i>Zanthoxylum leprieurii</i> Guill. & Perr.	Munyenye	October 2011	Mpigi

Note: \*Purchased from National Tree Seed Centre (NTSC).

species investigated in this study (Table 1) were selected from among multipurpose and medicinal plant species used for the treatment of HIV/AIDS (Nyamukuru 2013).

### 2.1. Sexual propagation experiment

Some seeds were collected from Mpigi District (31° 86' – 32° 43' E and 0° 16' S – 1° 19' N) with the help of TM practitioners while others were purchased from the National Tree Seed Centre (NTSC) of Uganda. The NTSC is 12 km from Kampala and ensures the delivery of quality tree seed to farmers. All seeds apart from *Milicia excelsa*, *Eucalyptus globulus* and *Markhamia lutea* were subjected to a pre-sowing treatment involving soaking in water at ambient temperature for 12 to 32 hours. One species, *Toddalia asiatica* was dipped in hot water at 90°C prior to soaking for 24 hours (Table 1). An equal number of seeds of each species was treated as a control, with no pre-sowing treatment. The number of seeds sown for each species depended on the number of seeds available at the time of sowing.

Seeds were sown in a mixture of lake sand and black soil in a ratio of 1:1 in a raised bed (10 × 1 m) under a shade net (73% screening factor). Although germination is defined as the emergence of the embryonic root (the radicle), in germination trials, the emergence of radicle through seed coverings is difficult to observe for buried seeds, and the emergence of the embryonic shoot (plumule) is usually taken to indicate germination (Forest Restoration Research Unit 2008). Therefore, in this experiment a seed was considered to have germinated upon the appearance of the plumule.

Seedlings were pricked out of the seedbed 3 weeks after germination, and transplanted into polythene pots (3 inch diameter) containing a medium of a mixture of black soil

and lake sand in a ratio of 3:1 and observed for growth for 97 days, still within the shade net. Seedlings were watered twice daily, in the morning and in the evening. Later, they were transferred from the shade net, hardened by exposing to gradually increasing sunlight and watered once a day for 14 days. Seed germination was recorded on a daily basis until no further germination was observed. The germination experiment was stopped when no new seeds had germinated for a consecutive 20-day period (Eyog-Matig et al. 2007).

### 2.2. Vegetative propagation experiment

The potential for asexual propagation was investigated by conducting a vegetative propagation experiment with stem cuttings of *Zanthoxylum leprieurii*, *Ficus saussureana* and *Warburgia ugandensis*. Cuttings were propagated in trays (100 × 50 × 15 cm), raised 10 cm from the ground. The sowing medium comprised a base layer of sieved lake sand, a middle layer of gravel and a top layer of sieved lake sand. *Ficus natalensis* and *W. ugandensis* were sown in a top layer of a mixture of lake sand and black soil in a ratio of 1:1. The experiment was conducted in a humidity tunnel (120 × 90 × 230 cm), placed under a shade net with 73% screening factor. A 1000 gauge transparent polythene sheet was used for the tunnel cover.

Cuttings (8–18 cm) were collected from stock plants in the morning (6.00 am) and kept in a cooling box to conserve moisture. They were planted the next day in the evening. Two or three leaves were cut halfway and left on the cuttings. Sixty stem cuttings each for *F. natalensis* and *W. ugandensis* and 46 each for *F. saussureana* and *Z. leprieurii* were planted in each treatment. Bases were squared using secateurs to avoid one sided rooting. Bases of half of the cuttings were treated by dipping in the rooting hormone (Seradix '2' 0.8% IBA) for 5 seconds

before setting in the planting medium to facilitate adventitious root formation. The remaining half acted as a control was not treated with hormone. Allocation of the cuttings to treatment was random. Excess of the rooting hormone was tapped off before planting in pre-wetted rooting substrate to a depth of 2 cm and at a spacing of 15 cm. The substrate was compacted around the base of the cutting for support. The fungicide (Agro-laxyl MZ 63.5 WP; Asiatic Agricultural Industries, Singapore) was used to control fungal infection.

An inspection was carried out every third day to remove fallen and infected leaves. Every 15 day the number of callused cuttings, rooted cuttings, roots per cutting, shoots, root length, dormant cuttings and degree of fungal infection was recorded. The experiment was terminated after 45 days from the time of planting.

### 2.3. Seedling growth experiment

The seedling growth experiment was carried out on *M. lutea* only. The seedlings were 8 weeks old at the time of the experiment and 180 seedlings of similar height were selected for the experiment. Half of the seedlings were treated with NPK (nitrogen, phosphorous, potassium mix) and the second half acted as a control and was not treated with NPK. Equal numbers of seedlings ( $n = 60$ ) were subjected to either of three watering treatments: watering daily, three times a week or twice a week. Collar diameter and height (cm) of all seedlings were measured at the beginning of the experiment and every 20 days thereafter for 80 days (i.e. four times).

### 2.4. Data analysis

The propagation potential from seed of each species was evaluated by determining its germination percentage (GP), mean length of seedling emergence time (MLE) the

number of days when 50% of the seeds have germinated – taken to be the measure of dormancy – and synchrony of germination. GP is considered high when 60% or more of the seeds germinate and low if less than 20% of the seeds germinate. The MLE is considered short if 50% of the seeds germinate within 21 days or less and long if they take 84 days or more. Germination is synchronous if all seedlings of a species emerge within 21 days, and highly asynchronous if they take more than 84 days (Blakesley et al. 2002). The Mann–Whitney U test was used to test for difference between GPs and mean length between treated and untreated seeds; the ANOVA test was used for differences between means of attributes of *M. lutea* seedlings; and the *t*-test was used for differences between means of lengths of roots developed on *F. natalensis* cuttings. All statistical tests were conducted using SPSS software v 16 (SPSS Inc., Chicago, IL, USA).

## 3. Results

### 3.1. Germination experiment

Germination for all species in the study occurred between 2 and 48 days, except for *Z. lepreurii* which failed to germinate in all treatments. Treatment by dipping seeds in either cool or hot water had no significant effect on the GP of all the species except *T. asiatica* (Mann–Whitney U;  $p > 0.05$ ; Table 2). *Toddalia asiatica* pre-treated with hot water failed to germinate and only those treated with cool water were germinated.

Species with the highest percentage of germination in control and treated seeds, having low dormancy and with synchronous or intermediate synchronous germination were *M. lutea*, *Psidium guajava*, *Spathodea campanulata* and *Maesopsis eminii* (Table 2). Stored seeds of *M. eminii* had lower GP and longer dormancy compared to freshly

Table 2. Germination trends in selected species. For details of the treatment see footnote of Table 1.

Species	GP (%)		MLE (days)		Synchrony of germination		Germination and synchrony category		Treatments
	T	C	T	C	T	C	T	C	
<i>Psidium guajava</i>	67	69	20	21	53	31	RG/LD/IS	RG/LD/IS	T2
<i>Maesopsis eminii</i>		72		34		74		RG/ID/IS	
<i>Spathodea campanulata</i>	33	37	11	15	15	36	IG/LD/S	IG/LD/IS	T2
<i>Maesopsis eminii</i> (stored)	57	32	41	44	88	73	RG/ID/AS	IG/ID/IS	T3
<i>Markhamia lutea</i>		24		8		14		IG/LD/S	
<i>Milicia excelsa</i>		4		17		56		LG/LD/IS	
<i>Eucalyptus globulus</i>		8		9		7		LG/LD/S	
<i>Entada abyssinica</i>	11	12	14	20	86	80	IG/LD/AS	LG/LD/S	T2
<i>Cordia Africana</i>	14	10	42	39	52	54	LG/ID/IS	LG/ID/IS	T2
<i>Canarium schweinfurtii</i>	10	11	50	78	43	72	LG/ID/IS	LG/HD/AS	T3
<i>Toddalia asiatica</i>	11	7	88	79	124	121	LG/HD/AS	LG/HD/AS	T3, T5
<i>Zanthoxylum lepreurii</i>									T1

Notes: Key: Germination percentage (GP), mean length of seedling emergence (MLE), RG = rapid germination, IG = intermediate germination, LG = low germination; LD = low dormancy, ID = intermediate dormancy, HD = high dormancy; S = synchronous, IS = intermediate synchrony, AS = asynchronous; T = treated, C = Control

T1 = Soaked in water at room temperature for 12 hours, T2 = Soaked in water at room temperature for 24 hours, T3 = Soaked in water at room temperature for 32 hours, T5 = Dipped in hot water (90°C) and left to soak for 24 hours.

harvested seeds. All other species had low GPs and were asynchronous. *Toddalia asiatica* and *Canarium schweinfurthii* were highly asynchronous. *Toddalia asiatica* was still germinating one year after sowing.

### 3.2. Vegetative propagation experiment

*Warburgia ugandensis* and *Z. leprieurii* cuttings did not sprout. *Ficus saussureana* and *F. natalensis* formed roots 15 days after setting. More roots were observed on *F. saussureana* cuttings treated with the growth hormone than in untreated cuttings, but the mean root length was similar between treated and untreated cuttings and very few shoots were formed. Fungi attacked all *F. saussureana* cuttings.

Almost all *F. natalensis* cuttings formed roots and shoots. Roots were significantly longer in untreated cuttings than in cuttings treated with rooting hormone ( $t$ -test<sub>0.144</sub>,  $p < 0.05$ ). Shoots in treated cuttings were longer than those which were not treated, but the difference was not statistically significant. Overall, it would appear that treatment with growth hormone does not confer any advantage for rooting and sprouting in *F. saussureana* and *F. natalensis* (Table 3).

### 3.3. Markamia lutea seedling growth experiment

Treatment with NPK led to a significant increase both in the diameter and height of *M. lutea* ( $p < 0.05$ ). Watering thrice a week was more effective at increasing diameter ( $p < 0.05$ ), but had no affect on height (Figure 1).

## 4. Discussion

The aim of this study was to improve the conservation of important medicinal and multipurpose species by providing information on propagation potential under different treatment regimes. The species, *P. guajava* and *M. eminii*, propagates readily from seed and can be easily managed by farmers using simple treatments and technology. The other species studied here have low GPs and are fairly asynchronous in their germination. *Toddalia asiatica* and *C. schweinfurthii* are difficult to propagate because they have very low propagation potential, as indicated by their low GPs, high dormancy and asynchronous germination. Asynchrony is an attribute that is not appreciated by farmers as they require seedlings that emerge at roughly the same time to facilitate efficient harvesting (Todd-Bockarie & Duryea 1993). It is recommended that further research be carried out on ways to shorten asynchrony in the seed species in this study.

*Zanthoxylum leprieurii* did not germinate at all, suggesting that the species is difficult to propagate from seed. The related species *Z. capense* also had little success in a South African study (Netshiluvhi 1996; Ramdas et al. 2012). The pretreatment used in this experiment, soaking in cool or hot water, had no significant effect on the germination of seeds of the selected species. Species that failed to germinate or those that had low scores on the germination parameters, suggesting low synchrony, probably require other techniques like immersion in sulphuric acid, to improve their germination (Todd-Bockarie & Duryea 1993).

Earlier efforts to propagate *W. ugandensis* and *F. saussureana* from cuttings were not successful (Al-Salem

Table 3. Growth response in cuttings of *Ficus saussureana* and *Ficus natalensis*.

	<i>F. saussureana</i>		<i>F. natalensis</i>	
	Treated	Untreated	Treated	Untreated
No. of cuttings with roots	15	1	30	28
Mean root length, cm, (range)	3.5 (0.3–17)	3.5 (1.3–3.6)	3 (0.2–79)	4 (0.2–56)
No. of cuttings with shoots (range)	3	1	23 (1–14)	27 (2–13)
Mean shoot length, cm, (range)			7.6 (0.2–31.5)	6.6 (0.1–25.8)
No. of cuttings with calluses	6	9	0	0
Fungal attack	All		0	3

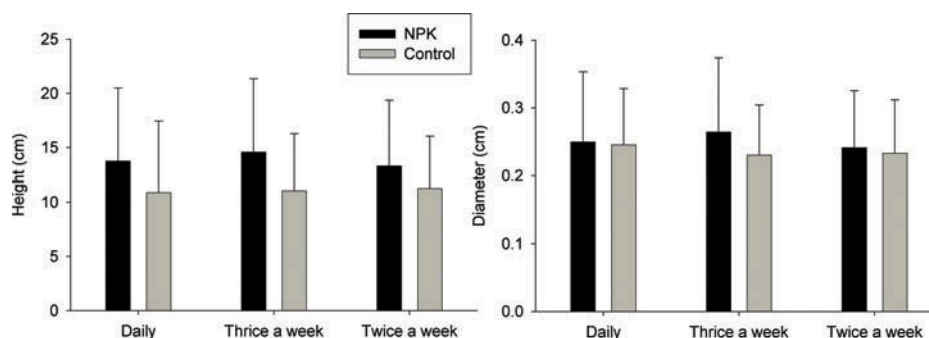


Figure 1. Influence of NPK and watering on height (a) and collar diameter (b) of *Markhamia lutea*.

2001; Akwatulira et al. 2011). *Zanthoxylum leprieurii*, *W. ugandensis* and *F. saussureana*'s failure to propagate may partly explain why these species are scarce in the wild and underscore the need for human intervention for their successful regeneration.

Recommendations for further studies should include experimentation with different treatments such as nicking and immersion in sulphuric acid, to find out if these treatments improve propagation rates. Nicking is especially recommended for small scale nurseries because of its low cost. Experimentation using tissue culture, especially for important but difficult to regenerate species like *Z. leprieurii*, would also benefit from further investigation.

Intermediate watering of *M. lutea* seedlings three times a week increases the growth by creating an increase in the girth without affecting height. Similar results have been observed in a seedling establishment study for *Merwillia natalensis*, where watering three times a week not only improved growth but also led to higher survival rates (Sparg et al. 2005).

## 5. Conclusions

The aim of this study was to determine the success of propagation and seedling establishment of selected medicinal and multipurpose tree/shrub species under different treatment regimes. The results suggest that the multipurpose species *P. guajava*, *M. eminii*, *S. campanulata* and *F. natalensis* can be propagated easily by farmers with simple technology, minimal water and no addition of fertilizer. Conversely, the medicinal species *Z. leprieurii*, *W. ugandensis* and *F. saussureana* could not be propagated in this study. *Markamia lutea* established readily with addition of fertilizer (NPK) and moderate watering (three times a week). This work contributes to a greater understanding of the requirements for successful propagation and domestication of important medicinal and multipurpose plants of Uganda.

## Acknowledgements

This study was funded by the Swedish International Development Agency (Sida) and the Inter-University Council for East Africa (IUCEA), through the Lake Victoria Research Initiative (VicRes) (Project No. 07/NR-01-10/002). Acknowledgments are due to Uganda National Council for Science and Technology (UNCST) for granting permission to conduct this study (Permit No. NS 373). Mr Daniel Mulindwa helped with the propagation experiments, Mr Ochwo of National Tree Seed Centre and Dr Sam Gwali of National Forestry Resources Research Institute (NaFORRI) are acknowledged for their technical advice on the propagation experiments. Makerere University provided additional logistical support. Dr Imogen Crawford and two anonymous reviewers are gratefully acknowledged for their useful comments to this manuscript.

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