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The effects of pretreatment of *Acacia nilotica* and *Acrocarpus fraxinifolius* seeds on germination and early development

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Abstract

A germination and early development study was performed in Musoma, Tanzania on the species *Acacia nilotica* and *Acrocarpus fraxinifolius* as part of an agroforestry research programme. Three pretreatments on the seeds were done; (1) no treatment, (2) soaked, and (3) scratched and soaked. Seeds from each treatment were sown in either shade or sun. Too few *A. fraxinifolius* seeds germinated to get reliable data. The scratched and soaked seeds of *A. nilotica* had a developmental advantage as they germinated earlier and more synchronized. Shade had a slightly positive effect on the germination time irrespective of treatment but acted negative on development. I conclude that scratching followed by soaking is preferable to small scale farmers as this is an easy and effective pretreatment of *A. nilotica* seeds. According to my study the germination phase of *A. nilotica* should be done in shade followed by full sun. This would give the best results for the farmer, but further studies need to be applied to fully establish this.

Introduction

Vi Agroforestry is an NGO (Non-Governmental Organization) that operates in the Lake Victoria basin, Eastern Africa with a vision of offering small scale farmers a sustainable environment and good living conditions. This vision is to be engrafted through agroforestry methods and corporation development (Nilsson, 2008).

Agroforestry is a land use system where trees and/or shrubs are grown together with crops and/or animals. Interactions between the different components are both ecological and economical (Ramachandran, 1993). Agroforestry systems enhance biodiversity, control erosion and the litter fertilize the soil. Together this makes agroforestry systems potentially sustainable (Sanchez, 1995). The agroforestry model is not just a sustainable farming system but also enforces the farmer's ability to gain food, medicine and firewood from their farms even during drought (Nilsson, 2008).

Acrocarpus fraxinifolius Wight & Arn. is a member of the Fabaceae family and has thin leaves that are decomposed fast. Its origin is in Asia but the species is vastly planted in Africa to give shade in tea and coffee plantations and also as a wind breaker (Maundu and Tengnäs, 2005). Vi Agroforestry programme recommends this tree as a substitute to farmers who wants to grow *Eucalyptus* ssp. with its relatively fast development (Nicholous Kabambo, 2011, personal communication). *Eucalyptus* trees deplete the soil with their high demand of nutrients. With their allelopathic compounds in litter they may inhibit the growth of other herbaceous plants which can lead to soil erosion and be a problem in the agroforestry system (Poore and Fries, 1985).

Acacia nilotica (L.) Delile is an indigenous tree species in Tanzania and has proven to be a tree with many useful qualities. It is suitable for firewood and fodder, and is used as medicine (Hines and Eckman, 1993). In addition, *A. nilotica* trees enter a symbiosis with nitrogen fixing bacteria giving the species soil improvement skills (Dreyfus and Dommergues, 1981).

The Vi Agroforestry programme has had some complaints from their associated farmers concerning poor germination rates after direct seeding of both *A. nilotica* and *A. fraxinifolius*. There have also been different opinions and advices regarding which pretreatment that should be applied on the seeds before sowing.

Many of the leguminous trees have dry seeds that are impermeable to water and pretreatment is therefore necessary for quick and reliable germination. This would give the seedlings a great advantage in the competition with weeds at direct seeding (Ross and Harper, 1972; Mandal, 1993). From the farmers point of view it is important that the pretreatment can be done without any advanced technical equipment and also that it is economically available for small scale farmers. Hot water treatments can be difficult to apply without thermometer and the risk is that the seeds die from being boiled. Several sources recommend treating the seeds with sulphuric acid (*A. nilotica*: Maundu and Tengnäs, 2005; Hines and Eckman, 1993; ICRAF are recommending it for both the species) but this is not feasible for most of the small scale farmers in Tanzania. Mechanical scarification followed by soaking have shown to be an effective way of getting rapid germination and increasing the

germinating rates in the leguminous trees *Leucaena leucocephala* and *Calliandra calothyrsus* (Mandal, 1993).

The germination phase and the first time is the most critical time period of a plants life, the seedling is vulnerable for dehydration, diseases and injuries (Raven *et al.*, 2005). Therefore it is of importance to study how shade will affect germination and early development of *A. nilotica* and *A. fraxinifolius*. This will tell us whether the species is suitable to sow in already shaded areas in an agroforestry system. Shade can be expected to benefit germination and seedling survival because of: Lower temperatures which results in less evaporation from the soil and seedling.

My hypotheses were:

- The pretreatment of scratching and soaking the seeds will facilitate the germination for both species, pretreated seeds will germinate earlier and within a shorter time span.
- Soaking without scarification will not have any effect when the seeds are irreversibly dried.
- If the scratched & soaked seeds germinate faster they will have a developmental advantage.
- The shade will keep the moist from the night a little longer which will save seedlings from drought stress and lead to a stronger development for those seedlings.

Materials and methods

Field experiment

The experiment was done within the agriculture training centre of Vi Agroforestry, 7 km south-east from Musoma in northeast Tanzania (coordinates -1.530600°, 33.855900° decimal degrees). The area had not been cultivated since Vi Agroforestry bought the land in 1993 but it has been grazed from time to time by passing cattle. The area is in a gentle slope and was covered with lower vegetation; graminoids and dwarf shrubs. The soil consists of loam to sandy clay loam (Kaihura and Kaboni, 2009). Before sowing all vegetation was removed and the sowing holes were prepared according to Vi Agroforestrys descriptions (Nicholous Kabambo 2011, personal communication). The uppermost foot of soil was mixed with one bucket (ten liters) of composted animal manure and placed back in the hole after that the second foot of soil had been dug up to be used as a micro catchment, a half circle facing the slope to collect rainwater (figure 1). The holes where arranged in four rows with 12 holes / row having 2 m between each hole and row. The *A. fraxinifolius* field was higher up in the slope with a small newly cultivated maize field above it. Below this a large maize *Zea mays* L. field was established and below that the *A. nilotica* field begins.

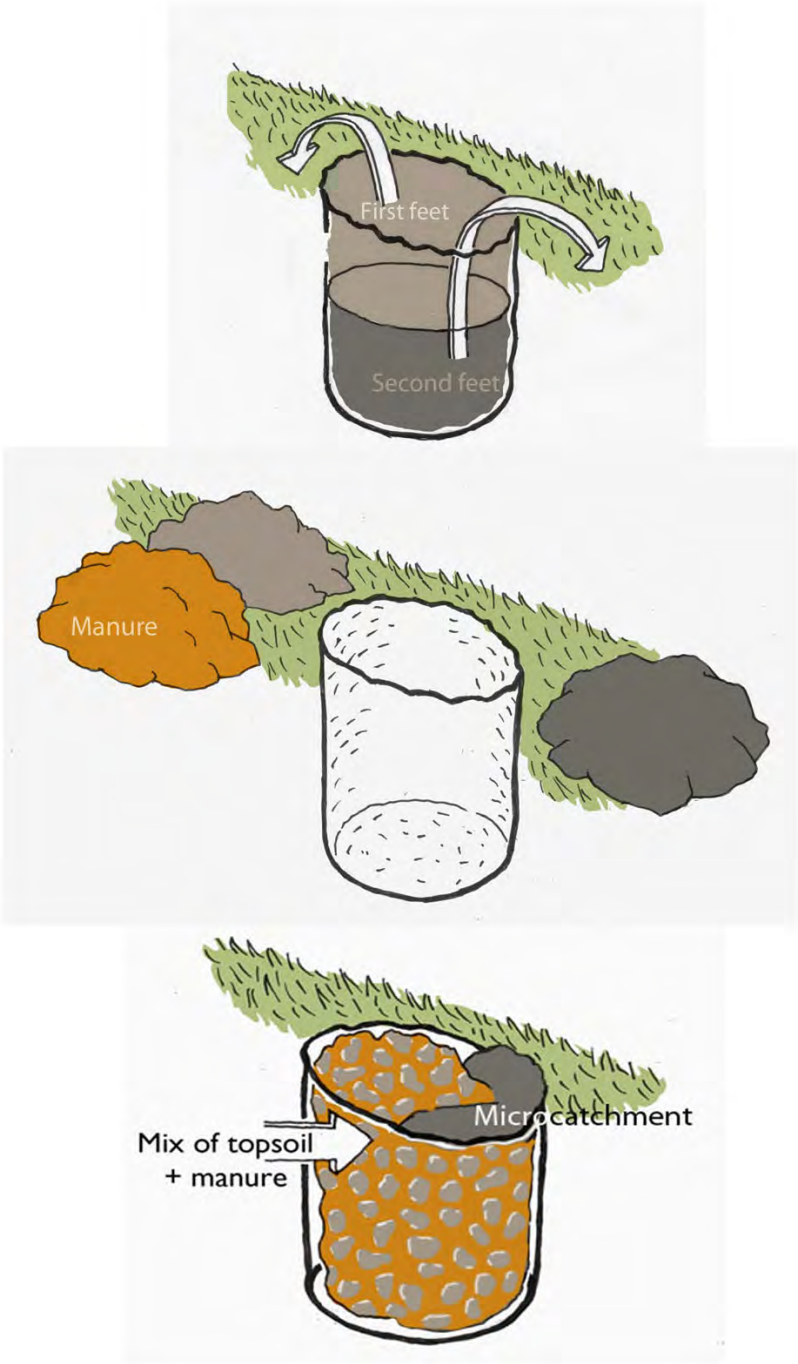

Figure 1. The procedure of preparing seeding holes following Vi Agroforestry's protocol. The first foot of soil in the hole is digged up and mixed with one tin (ten liters) of composted manure. The second foot in the hole will become a microcatchment. The microcatchment is a halfmoon shaped bank of the soil facing the slope to catch some of the rainwater. The mixture of the topsoil and manure is then placed back in the hole and seeding can begin. Illustration Sven Holmberg

Figure 2. The experiment in Musoma Tanzania 2011 on pretreatment of *Acacia nilotica* seeds. One shaded sowing hole with three out of five possible seedlings. Note the bank of soil at the right; the microcatchment. *Photo Siri Holmberg*

300 seeds of both species that had been stored in darkness and in a temperature about 16°C for two years, were treated in three different ways with 100 seeds in each treatment:

- No pretreatment.
- Soaked in room tempered tap water for 18-21 hours. At sowing one third of the soaked seeds were swollen, a sign that the seeds had been imbedded i.e. the initial step in germination (Raven *et al*., 2005).
- Scratched against glass paper (grain size 80) through the outer layer for *A. nilotica* and for *A. fraxinifolius* until there were scratches in the outer layer. The scratched seeds were then soaked in room tempered tap water for 15-17 hours. At sowing most of the *A. nilotica* seeds were swollen and for *A. fraxinifolius* at least half of the seeds were swollen.

The seeds were then sown, at the 8th of November for *A. nilotica* and the 10th of November for *A. fraxinifolius,* in the shape of a rectangular with one seed in each corner and one in the middle, all five seeds from the same treatment. The *A. nilotica* field was watered after sowing as the soil was totally dry, for *A. fraxinifolius* it had newly rained so no watering was needed. Throughout the time of the study it rained every second or third day so no further watering was needed. Which treatment that should be seeded in which hole was randomized before sowing. At half of the holes the sowing area was shaded from early morning sun until midday. The shade was made of a rectangular black plastic sheet 30 cm x 40 cm, attached to two sticks and placed 20 cm from the nearest seed (figure 2). The seeding holes were weeded

when needed.

Germination was recorded as the earliest sign was visible, namely a small stack of soil with typical cracks in it. The development was recorded as the number of leaves excluding cotyledons. The recording took place every second day starting from day four from the seeding day up till day 33 for *A. fraxinifolius* and 35 for *A. nilotica* with a break of two visits at day number 30, 32 and 32, 34, respectively.

Statistical analyses
To analyse treatment and exposure effects on germination day I used a two-way analysis of variance, with three levels of treatment and two levels of exposure. The analyses on development were done using germinated seeds for those seedlings that survived the whole experiment. I analyzed the number of leaves using repeated measures analysis with treatment and exposure as fixed factors, and a two-way ANOVA at day 35. All analyses were done using IBM SPSS statistics 21 followed by Tukey post-hoc-test with 0,05 as the significance level.

Results

During the experiment there were several heavy rainfalls and as the area was situated in a slope, soil erosion became a problem. In the gravest cases the seeding holes got an additional 10 cm layer of soil and that caused the death of some of the seedlings. The *A. fraxinifolius* sowing area also had some problems with drainage, which resulted in that some of the seeding holes had standing water in them.

Acrocarpus fraxinifolius
The extension on the disturbances of the *A. fraxinifolius* area due to erosion and drainage was not recorded.

In total, the *Acrocarpus fraxinifolius* germination rate was 5% (15 seeds) and had a mortality of 47%. No further analyses were done.

Acacia nilotica
The percentage of disturbances in the *A. nilotica* field due to erosion:
The control group got 7 % of the sowing holes affected.
The soaked treatment got 10 % of the sowing holes affected and 1 seedling died due to erosion.
The scratched and soaked treatment got 14 % of the sowing holes affected and 5 seedlings died due to erosion.
Totally ca 10 % of the *A. nilotica* experiment was affected.

Germination
For *A. nilotica* the germination rate was in total 63% (188 seeds), 59, 66 and 63 % germinated in the three treatments respectively; control, soaked and scratched and soaked. The mortality was in total 5%, ranging between 2-6% for the different treatments. For the numbers, see table 1.

Table 1. The number of germinated seeds of *Acrocarpus fraxinifolius* and *Acacia nilotica*, and number of seedlings that died during the experiment. The seeds had gone through one of the three treatments; Control, Soaked or Scratched & Soaked. The treatment groups where then grown in different light exposure; shade or sun. *N* = 50 for each treatment/exposure combination and species, in total *N* = 600.

		Acrocarpus		Acacia	
Treatment	Exposure	Germinated	Died	Germinated	Died
Control	Shade	2	0	31	0
	Sun	0	0	28	2
Soaked	Shade	0	0	31	1
	Sun	1	0	32	1
Scratched & Soaked	Shade	5	3	33	3
	Sun	7	6	33	3

For *A. nilotica* there were significant differences in germination date between treatments (table 2); seeds that were scratched and soaked germinated earlier, almost in half the time compared to the other treatments, and the germination was more synchronized (figure 3). There was no interaction between light and treatment (table 2) but light had a significant effect irrespectively treatment (table 2). Figure 3 shows that shade gives a slightly earlier germination.

Table 2 The effect of treatment and exposure on germination day of *Acacia nilotica,* values presented are df, F and significance, two-way analysis of variance.

Source	**Df**	**F**	**P**
Treatment	2	87.326	$< 0.000^{***}$
Exposure	1	6.768	0.010^{**}
Treatm. × Exposure	2	0.481	$0.619^{n,s.}$
Error	182		
Total	188		

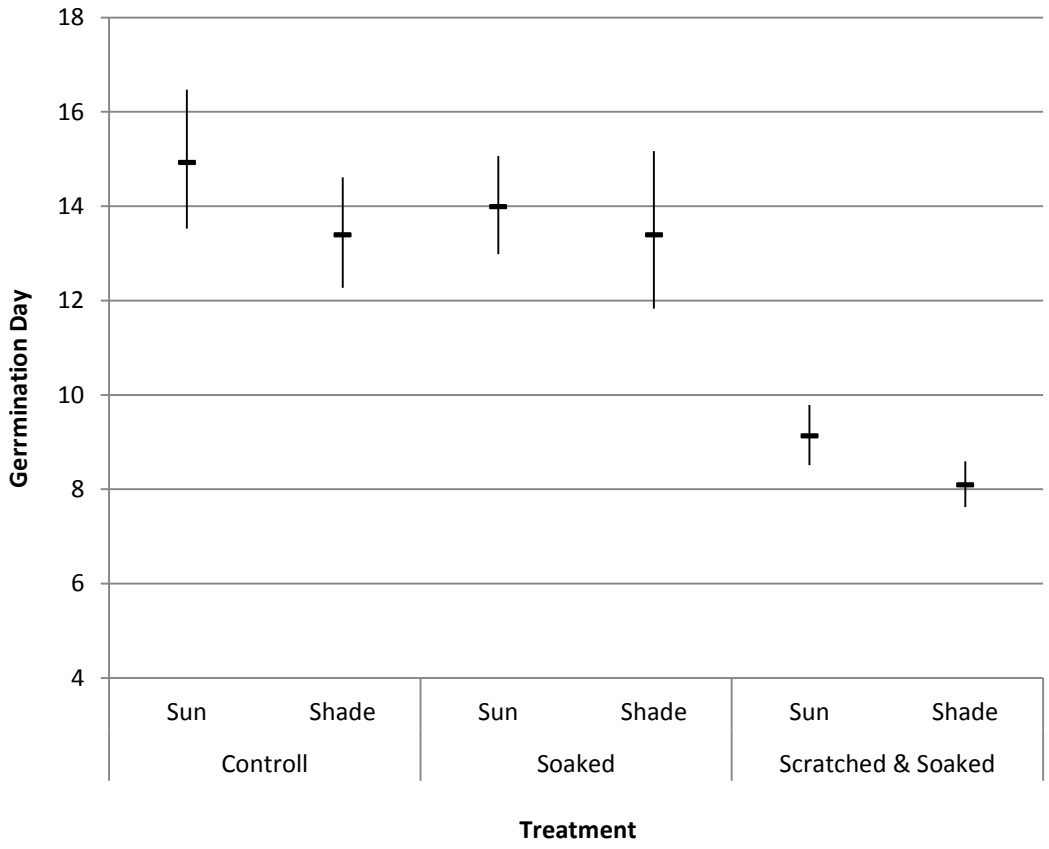

Figure 3 Germination day (average ±95% Confidence Interval) for the different treatments in the exposure of light or shade. The figure is showing that the scratched & soaked treatment did have an earlier and more synchronized germination

Development

The treatment and light had a significant effect on number of leaves but the interaction between the two was not significant (table 3). The scratched and soaked treatment gave seedlings with a stronger development (Tukey test) (figure 4). Seedlings that had been grown in full sun had a stronger development (figure 5)

Table 3 The effect of treatment and exposure on the development of *Acacia nilotica* seedlings, values presented are df, MS, F and significance, repeated measures analysis of variance.

Source	df	Mean Square	F	P
Time	1.492	98093	1503.931	0.000***
Time × Treatment	2.984	732.227	11.226	0.000***
Time × Light	1.492	255.916	3.924	0.032*
Time × Treatment × Light	2.984	27.05	0.415	0.742n,s.
Error(Time)	250.65	65.225		

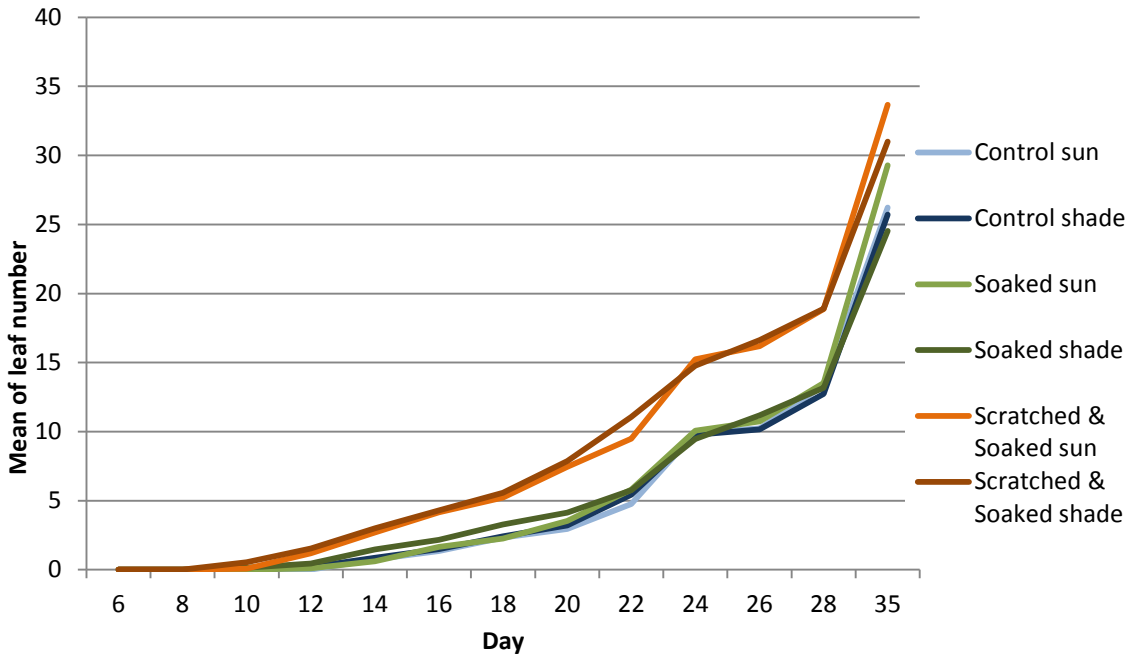

Figure 4 Development measured in number of leaves (mean values) of *Acacia nilotica* seedlings for the different treatments and exposures through time.

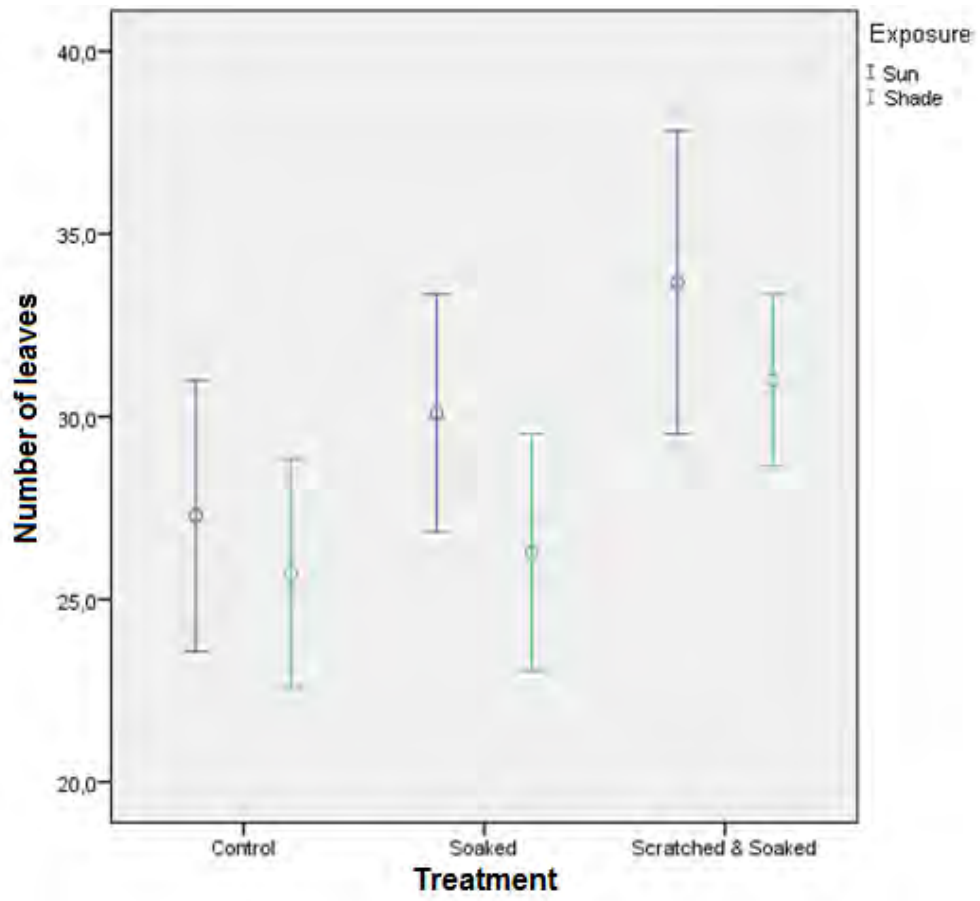

Figure 5 Number of leaves of *Acacia nilotica* seedlings at day 35 for the different treatments and exposures. Values are means ± 95 % confidence intervals.

Discussion

Acrocarpus fraxinifolius

Under natural conditions *Acrocarpus fraxinifolius* seeds can germinate from the first week up to a year after sowing (ICRAF). Few of the seeds germinated at the same time and few at all germinated within the 33 day period. This poor germination could be because the scratching of the surface was not severe enough. At sowing half of the scratched and soaked seeds were swollen whereas among the seeds that were just soaked a third were swollen.

That very few of the imbibed seeds germinated may indicate that there are other factors that are playing a major role in the germination of *A. fraxinifolius* in addition to imbibition. Another possibility would be that the seeds were dead or died before any visible signs of germination due to insufficient drainage and/or erosion. After some heavy rains during the experiment some of the seeding holes were covered with standing water and some of the holes were covered with up to 10 cm of soil that had been flushed down the slope.

Due to too small data series it is impossible to conclude whether shading had any effect on the germination and development of *A. fraxinifolius.* According to literature *A. fraxinifolius* is a light demander (ICRAF) but more research is needed to finally establish this.

Acacia nilotica

For the scratched and soaked treatment most of the seeds were swollen after the soaking. This shows that the scratching does facilitate the imbibitions and gives an advantage in the germination process; the mean value of the germination day was almost cut by half compared to the other treatments (figure 3). This is accordance with Mandal and Nielsen (2004) were they scratched and soaked the hard coated seeds of the leguminous *Calliandra calothyrsus*. In 1993 Mandal did a similar study with *C. calothyrsus* and *Leucaena leucocephala* were he also came to the conclusion that scarification performed the earliest germination.

Ross and Harper (1972) have shown that the growth rate of individual seedlings is negatively affected by the density of earlier germinated seedlings. In a tough competition just a few days of delayed germination can dramatically reduce biomass. This tells us that early germination is an ecological advantage in the competition for space, light, nutrients and water against weeds. Of course, this is also advantageous for the farmer.

For the soaked seeds, one third were swollen after soaking which means that those seeds had not been irreversibly dried or had got their seed coat damaged before the study. There was no significant difference in germination day or numbers of germinated seeds between the control and the soaked treatment. This may point to that the seeds were dead, otherwise the soaked seeds would have an advantage like the scratched and soaked ones and no such advantage compared to the control was recorded. Seeds that are not irreversibly dried may not survive two years of storage. However, it is unlikely that the vitality of the seeds in the soaked treatment group would differ from the vitality of the seeds in the other two treatment groups.

There was no significant difference in numbers of germinated seeds between the different treatments even though almost all of the scratched and soaked seeds were imbibed, which is the initial step in germination. That leaves us with three alternative explanations:

- 40 % of the seeds were dead, that is about the same number as for the seeds that were only soaked and became swollen (see above) and seems accurate. The seeds could also/or have been damaged by oxygen deficit (Bewley *et al.* 2006). Mandal and Nielsen (2004) state that only eight hours of soaking is safer after scarification.
- 40 % of the seeds did germinate but died before showing any visible signs.
- There are other factors that affect germination.

It can also be a mix of all three possibilities but more research is needed to draw any conclusions.

That the development of the seedlings and the germination is more synchronized in the scratched and soaked treatment is benefitting for the farmers in addition to the early germination. The farmer can easier follow the seedlings and can weed and build protection for all the seedlings at the same time and does not need to wait for the right timing for each plant. It is also easier for the farmer to know which seeds that were viable so that re-seeding can be done.

The reason why the seeds in the control and soaked treatment were less synchronized in their germination is probably due to differences in physical processes that roughs the seed coat, or differences in the microbial activity. As a consequence the imbibition starts at different occasions and germination then becomes spread over time (Lambers *et al.,* 1998).

That the development was significant at the repeated measures analysis does not necessarily mean that the scratched and soaked treatment actually gives a faster development rate. My conclusion is rather that the earlier start was the factor giving them an advantage. Figure 4 shows that the seedlings development of the scratched and soaked treatment is more or less parallel with the other two treatments. It seems like the other treatments will catch up later on.

Shading did have an effect on both germination and development. Shading had a positive effect on germination, the seeds germinated earlier. On the other hand, shading had a negative effect on development, as seedlings developed fewer leaves. Chaudhry *et al*. (2004) did a study of shading *A. nilotica* sowings and found 33 % germination for those in shade vs. 84,6 % in direct sunlight and it took about twice the time for those who germinated under shading compared to the sun lighted ones. This differs from the results in my study. The seeding holes were shaded in the early morning hours unto midday, which could have the positive effect that the soil did keep more moist that facilitated the germination. But after midday the seeding hole was in full sun unto sunset. Maybe there is a critical point where the dominance from advantages of shade shifts to the disadvantages.

In shaded conditions seedlings allocate resources differently and tend to elongate the stem and shoot growth at the expense of root development, which leads to a more vulnerable seedling with less ability of water uptake (Kramer and Kozlowski, 1979 cited in Chaudhry 2004). This could be devastating for the seedling when species specialized for dry zones tend to form a well developed root system before height

growth begin (Schmidt, 2000). ICRAF describes *A. nilotica* as a strong light demander and that the seedling needs full sun. My results confirm this. It would have been interesting to find out if the mortality of the shaded seedlings would have increased, compared to those in sun, during a dry season. This would have empowered my reasoning that the shaded seedlings have developed a smaller root system and would thus have severe problems covering the plant's need for water.

A problem that both sowing areas experienced was erosion. After rain some of the holes got buried under almost 10 cm of soil. The erosion caused some of the mortality and can also have influenced germination insofar that the seedling did not have enough energy to emerge through the extra soil and that germination therefore never was recorded. It could also be that the oxygen rates or light levels became too low, both aspects can have effect on the initiation of the germination in seeds (Lambers, *et al.* 1998).

Conclusion and future projects

If early germination and steady development is to be achieved for *A. nilotica* the scratched and soaked treatment should be applied. According to my study the germination phase of *A. nilotica* should be done in shade followed by full sun. This would give the best results for the farmer, but further studies need to be performed to fully establish this. For *A. fraxinifolius* further studies have to be done to find a reliable way of propagating the species.

It is known that *Acacia nilotica* enter into a symbiosis with nitrogen fixing rhizobium bacteria (Dreyfus and Dommergues, 1981). It would be interesting to investigate if the propagation of *Acacia* seeds would benefit from being sown in soil where rhizobium bacteria are present. Soil from underneath a *Leucaena leucocephala* could be suitable because this species enter symbiosis with the same type of rhizobium strain as A*. nilotica* (Dreyfus and Dommergues, 1981).

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Keywords

Agroforestry, Tanzania, Vi Agroforestry, imbibition, scarification