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ABSTRACT

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1. INTRODUCTION

Reducing emissions from deforestation and forest degradation, and enhancing forest carbon stocks in developing countries (REDD+) is a mechanism for climate change mitigation and this can be done through reforestation, agroforestry and natural forest regeneration. The distinction between agroforestry and assisted natural regeneration (ANR) is not always clear because agroforestry is a collective term that describes land use systems and practices that integrate trees with crops and/or animals on the same land unit while ANR is natural forest regeneration with human assistance intended to encourage the natural regeneration of native species at a site. These restoration activities can be undertaken on degraded landscapes and abandoned cropland (fallow).

One of the common impediments to artificially regenerating trees on degraded dry tropical forest sites is seed dormancy which occurs due to some chemical, physical and/or physiological traits in seeds that cause regulated germination which is believed to be a survival mechanism that ensures that germination occurs only when environmental conditions are favorable [1]. Physical seed dormancy occurs in a number of species in the Fabaceae family and seed scarification has been reported to give the highest seedling emergence rate and the most vigorous seedlings [2]. The majority of studies on seed dormancy in African dry tropical woody species have focussed on the seedling stage [3]. African dry forest tree species under go three post-seed phases during regeneration: seedling, sapling and tree phases. I define these life history stages as follows: (i) seedling as a plant that is less than 1-year old that is recognized by the presence of cotyledons, (ii) sapling as a plant that is >1.0 year old, <2.0 m tall and with a diameter at breast height (1.3 m above-ground, dbh) of <3.0 cm that originated from a seed, (iii) tree as a plant that is >1.0 year old,

>2.0 m tall and with a dbh of >3.0 cm. The transitions from seedling to sapling to tree have been a subject of many studies and there is growing support for the idea that demographic constraints in seedling recruitment and sapling release are responsible for much of the variability in tree density in savanna ecosystems [4].

Whereas seed treatment enhances seedling recruitment the long-term effects of this procedure on saplings and trees have rarely been investigated, especially in agroforestry, restoration and carbon sequestration projects [5]. This study aimed at investigating the effects of seed scarification on the demography and growth of planted *Senegalia* (formerly *Acacia*) *polyacantha* Willd. trees in comparison to non-planted trees over a period of 21 years (1996 – 2017) at a site that was initially degraded by charcoal production and later briefly cultivated in central Zambia. The investigation aimed at assessing the effects of seed scarification on (i) seedling emergence and survival and (ii) growth and longevity of *S. polyacantha* trees. The findings of the study have implications for using this species in agroforestry, forest restoration through natural regeneration and carbon sequestration projects in Sub-Saharan Africa where this species occurs.

2. MATERIALS AND METHODS

2.1 Study site

The 0.80-ha study plot is located at 15.467° S, 28.183° E, 1260 m altitude above sea level (asl), about 15 km south of Lusaka city in central Zambia.

The plot was established in 1996 when it was divided into two subplots: control (0.31 ha) and experimental (0.49 ha). The control subplot is split into three blocks while the experimental subplot is split into nine blocks of variable sizes (Figure 1) due to presence of termite mounds and the layout required that each mound should not extend beyond a single block. There are five termite mounds at the plot (one each in ES, EN, WCC, WC and WN). Trees in the area had been selectively cut for charcoal production during 1992 – 1994. Charcoal production was by the earth-kiln method that removes about 93% of the aboveground wood biomass while the rest remains in residual uncut trees (Chidumayo, 1991). At the time of plot establishment residual trees on the plot consisted of *Piliostigma thonningii* (Schumach.) Mile-Redhead, *Senegalia polyacantha* Willd. and *Vachellia sieberana* DC. The plot was fenced off in 1994 to keep out livestock and to minimize undesirable human disturbances in the re-growing woodland as part of measures for assisted natural regeneration.

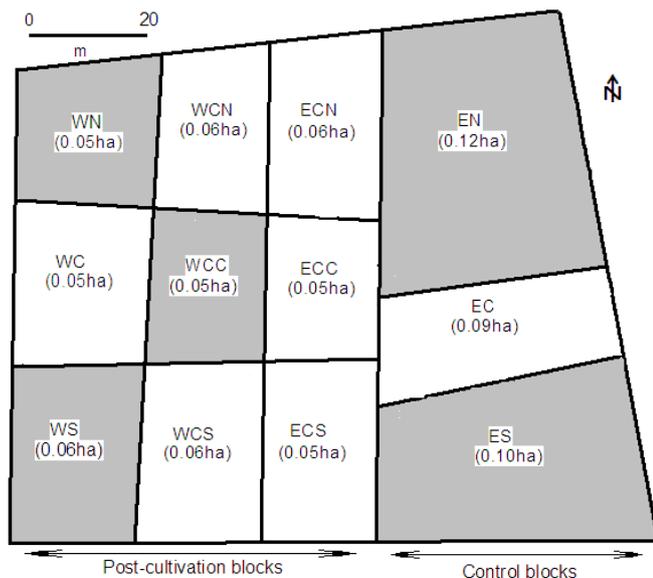


Figure 1: Layout of blocks at the study plot. Blocks are named according to their location on the plot: W for west, E for east, S for south, N for north and C for center. Annually burnt blocks are indicated by grey shading.

The soil at the study plot is predominantly sand clay loam with 47% sand, 34% clay and 19% silt and a pH of 5.4 [5]. The climate at Mt Makulu (15.550°S, 28.267E, 1240 m asl), 13 km south of the study plot, is subtropical with alternating dry (May – October) and wet (November – April) seasons with a long-term (1970 – 2017) annual mean ($\pm 1se$) precipitation of 836 ± 34 mm with a coefficient of variation of 27.5%. Annual mean minimum and maximum temperatures at Mt Makulu are $14.7 \pm 0.08^\circ\text{C}$ and $27.6 \pm 0.12^\circ\text{C}$, respectively.

2.2 The study species

Senegalia polyacantha occurs in Southern, Eastern and West Africa and produces dehiscent pods that split when ripe and pod-valves, with their attached seeds, are dispersed by wind. The seeds weigh (mean $\pm SE$) 0.096 ± 0.002 g. In Zambia *S. polyacantha* can reach a height of 18 m and a breast height diameter of 40 cm [6]. *Senegalia polyacantha* is also a fast-growing nitrogen-fixing tree that has been used as an agroforestry and restoration species in Sub-Saharan Africa [7-9]. Under natural conditions it is considered as a secondary succession species that invades disturbed landscapes [10,11].

2.3 *Senegalia polyacantha* enrichment planting and crop cultivation

The experimental subplot was subjected to tree enrichment planting with *S. polyacantha* in December 1996. Tree enrichment planting involved nursery-raised transplants and seed sowing in north – south rows with an inter-row and inter-plant interval of 2 m, except on positions with already existing naturally established woody plants where no planting was done. Sown seeds and all nursery-raised seedlings were from scarified seeds, intended to reduce seed coat dormancy, that were collected from trees in the neighbourhood of the study site. Enrichment planting rows were numbered from west to east and rows 1 – 3 were planted with 108

transplants with row 1 covering blocks WS, WC and WN and rows 2 – 3 covered blocks WCS, WCC and WCN. Sixty seeds were sown in rows 4 – 5 that covered blocks ECS, ECC and ECN. The survival of plants from seed sowing and nursery transplants was monitored annually until 2017. The experimental subplot was cultivated during three seasons (1996/97 to 1998/99) by intercropping maize with pumpkin and beans and hand hoeing and weeding but care was taken when cultivating to ensure that no damage was done to all established tree seedlings, saplings and live stumps with sprouts. All cultivation in the experimental (here after post-cultivation) blocks ceased in 1999 while blocks in the control subplot were never cultivated following the initial disturbance caused by charcoal production.

During the 1997 – 1999 period all blocks in the experimental subplot were protected from fire, except for an accidental fire in July 1998 that affected blocks ECN, WCN, WN, WC, WCC and ECC. Blocks EN, ES, WS, WN and WCC totally 0.38 ha were annually burnt from 2000 while the rest of the blocks totally 0.42 ha were fire protected (see Figure 1). To ensure effective fire control, a 3-m firebreak surrounding each block was annually cleared of any herbaceous material in April using hand hoes. Although the precise timing of burning depended on the time when the seasonal rains ended, burning was done any time from mid-June to mid-August that corresponds to the early dry season burning [12].

2.4 Seedling germination and survival

In order to investigate the effect of seed scarification on seed germination and seedling mortality 385 untreated and 144 scarified *S. polyacantha* seeds, collected from trees in the neighbourhood of the study plot, were sown along the firebreak on the eastern perimeter of the plot in November 2003. Untreated seeds were sown on 77 stations with five seeds per station and scarified seeds were sown on 72 stations with two seeds per station in two separate rows. The stations and rows were 1.0 m apart and the planting stations were marked by aluminum stakes for easy re-sighting during subsequent inspections [13]. Seeds were sown in shallow holes (2 – 3 cm deep) and seedling emergence (used as a proxy for seed germination) was monitored at weekly intervals during the wet (rainy) season for three years to obtain data on phased seed germination. A census of surviving seedlings was conducted at the end of the first year in December 2004 to determine seedling mortality.

2.5 Tree and sapling data

Censuses of saplings, as defined above, among planted and non-planted plants were conducted biannually from 2006 to 2016 in all blocks. The location of each tree at the study plot was mapped for easy re-sighting and the breast height (1.3 m above ground) diameter (dbh) measurement point marked by permanent paint. At the time of first measurement or time of recruitment the source of each tree was recorded under three categories: sown seed, nursery transplant and non-planted. The dbh (cm) of each tree, as defined above, was measured to the nearest mm and recorded in March or April of each year until 2017. Tree measurements started in 2001 after adequate tree recruitment was evident. Tree mortality was estimated from annual censuses data in the blocks that were conducted during tree measurements.

2.6 Data analysis

Seedling emergence rate and mortality between untreated and scarified seeds were compared using the two proportions Fisher's exact test (Z) at $P = 0.05$ significance level. Census data for trees recruited in 2001 were analyzed using survival analysis and significance of differences between planted and non-planted trees and between fire protection and annual burning treatments were determined by the logrank (L) test at $P = 0.05$. The logrank is a non-parametric test for comparing two survival distributions using censored data [14]. Tree growth rates were calculated on an annual basis by subtracting previous year dbh from current year dbh of each tree and the non-parametric Kruskal-Wallis One-way Analysis of Variance (AOV) test (H) at $P = 0.05$ using ranks was applied to dbh increment data to determine the significance of differences in tree growth rates between planted and non-planted trees and between fire protection and annual burning treatments.

In order to determine factors (explanatory or predictor variables) that might explain variations in growth rates, tree dbh increment data were subjected to best subset regression analysis in Statistix 9.0 [15]. using three predictor variables: year, tree density and mean tree size. Regression analyses were carried out in two phases. Firstly, the best subset regression analysis was carried out to select predictor variables that explained the largest variation in tree growth rate [16,17]. When two independent variables are highly correlated the analytical procedure used automatically drops one of the predictor variables to avoid problems of collinearity (Analytical Software, 1985–2008) [13,18]. Best subset regression analysis simultaneously compares models with single variables and all their possible combinations. The model with the lowest Akaike's Information Criterion (AIC) for small samples (AICc) was selected as the best model (Burnham & Anderson, 2002) [18]. However, after executing ordinary linear regression analysis for the best model, any predictor variable that had a high variance inflation factor (VIF >10.0) was excluded from the model because in a multiple regression this indicates a problem with collinearity [19].

3. RESULTS

3.1 Seedling emergence and survival

Out of 144 scarified seeds a total of 74 seedlings were observed while among the 385 untreated seeds a total of 122 seedlings were realized. The seedling emergence rate of 51.4% among scarified seeds was significantly higher than that of 30.2% among untreated seeds ($Z = 4.07, P < 0.0001$). Seedling emergence from scarified seeds occurred within three weeks of sowing and no new seedlings were observed after this period. In contrast seedling emergence occurred over two wet seasons among untreated seeds. During the first season 91.8% of the 122 seedlings were recorded over a period of seven weeks while 3.3% emerged between 13 – 14 weeks after sowing. A further 4.9% of the seedlings emerged during the second wet season and no seedling emergence was observed in the third season. Seedling emergence rate from the 60 scarified seeds sown in 1996 in blocks WCS, WCC and WCN was 63% (38 seedlings).

Among the 2004 cohorts from seeds sown in November 2003 mortality during the first year of 50% among seedlings from scarified seeds was significantly higher than that of 31% among seedlings from untreated seeds ($Z = 2.47, P = 0.01$). Proportionately significantly more seedlings from untreated seeds (69%) survived during the first year than the 50% from scarified seeds ($Z = 2.47, P = 0.01$). Mortality during the first year among seedlings from scarified seeds sown in 1996 in blocks WCS, WCC and WCN was also 50% as observed for the 2004 cohort.

3.2 Tree recruitment and survivorship

Planted trees were recruited in 2001 but there was a punctuated but continuous recruitment among non-planted plants with recruitment occurring from 2001 to 2006 and 2008 to 2013 for annually burnt and fire protected blocks, respectively (Figure 2). Of the 44 trees from the planted stock 16 originated from sown seeds and 28 from nursery transplants. Thus among sown seeds 19 (50%) and three (8%) died as seedlings and saplings, respectively, and 16 (42%) transitioned into the tree phase. Out of the 108 nursery transplants 28 (26%) transitioned into the tree phase, 42 (39%) died as saplings while 38 (35%) were still live saplings in 2017 when the study ended. There was no significant difference in the proportions of plants recruited into the planted tree population between transplants and seedlings ($Z = 1.6, P = 0.10$). In spite of the punctuated tree recruitment in the non-planted population, censuses of non-planted saplings revealed that these were present in adequate numbers that exceeded the tree population throughout the study (Figure 2).

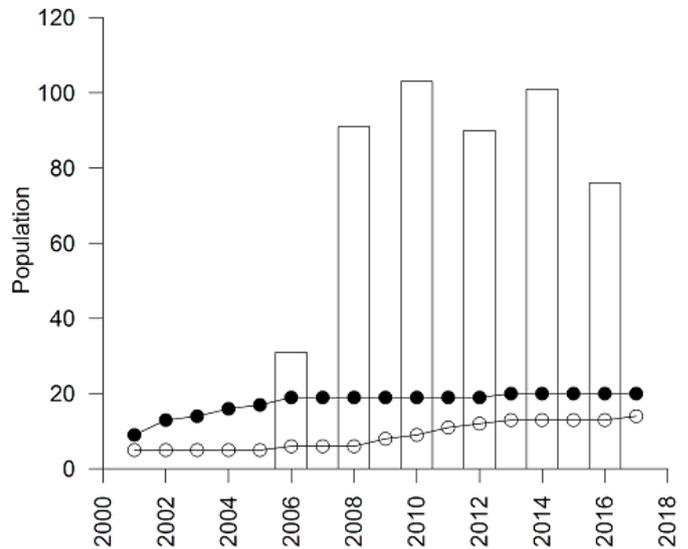


Figure 2: *Senegalia polyacantha* non-planted tree population in fire protected (empty circles) and annually burnt (filled circles) blocks and non-planted sapling population (empty bars) at the study plot.

In 2001 a total of 14 and 44 trees were recruited into the population among non-planted and planted trees, respectively. The survival of these sub-cohorts was followed during the study (2001 – 2017). Tree deaths were first observed six to seven years after recruitment but over half of the planted trees had died 12 years after recruitment compared to seven percent among non-planted trees (Figure 3).

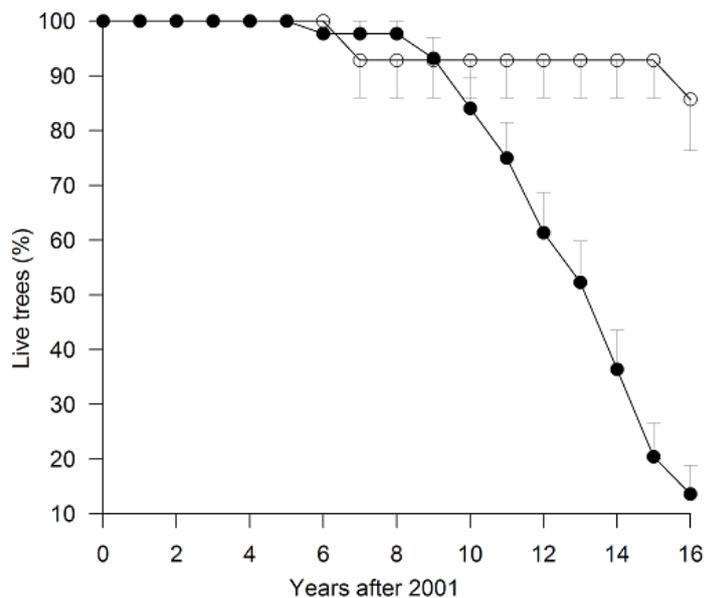


Figure 3: Survival distribution patterns for planted (filled circles) and non-planted (empty circles) *Senegalia polyacantha* trees recruited into the population in 2001 at the study plot. Vertical lines show standard error of mean and are shown in one direction for clarity.

By the end of the study in 2017, 86% of the planted trees had died compared to 14% among non-planted trees. Survival analysis revealed significant differences in the survival distribution patterns between planted and non-planted *S. polyacantha* trees ($L = 4.74, P < 0.0001$). Among the planted trees there was no significant difference in the survivorships of trees from transplants and seed ($L = 1.57, P = 0.12$). Similarly there was no significant difference in the survivorships of trees under fire protection and annual burning ($L = -1.14, P = 0.25$).

Trees at time of death had a mean dbh of 7.3 ± 0.6 cm for the planted population and 13.9 ± 2.5 cm for the non-planted population. To determine if tree death was associated with a gradual decline in growth rate, changes in diameter increment for the previous five years prior to death were

analyzed. There was a weak but significant negative linear relationship between year before death and annual dbh increment: $y = -0.105 - 0.11x$, $r^2 = 0.05$, $P = 0.007$ (Table 1). This suggests that tree senescence was gradual with growth rate declining over several years prior to death.

Table 1: Pattern in diameter increment of *Senegalia polyacantha* trees prior to death at the study plot.

| Sample trees | Year before death | Diameter increment (mean±1se) |
|--------------|-------------------|----------------------------------|
| 28 | -5 | 0.46±0.14 |
| 29 | -4 | 0.43±0.17 |
| 30 | -3 | 0.12±0.12 |
| 30 | -2 | 0.06±0.10 |
| 30 | -1 | 0.08±0.10 |

3.3 Tree growth rates

In 2017 planted trees had a mean dbh of 13.7±1.1 cm compared to 17.5±1.5 cm among non-planted trees and the difference was significant ($t = 2.03$, $P = 0.05$). There was a significant difference in the annual growth rate of planted and non-planted *S. polyacantha* trees ($H = 44.17$, $P < 0.0001$; Figure 4a). Overall planted trees had a dbh increment of 0.22±0.03 cm yr⁻¹ compared to 0.56±0.05 cm yr⁻¹ for non-planted trees. However, two distinct periods of differential growth for each group of trees were evident: (i) pre-2010 (2002 – 2009) and (ii) post-2009 (2010 – 2017). Among planted trees annual dbh increment was 0.29±0.04 cm yr⁻¹ for the pre-2010 period compared to 0.04±0.06 cm yr⁻¹ for the post-2009 period and the difference was significant ($H = 19.47$, $P < 0.0001$). For non-planted trees the dbh increment of 1.01±0.07 cm yr⁻¹ for the pre-2010 period and 0.30±0.05 cm yr⁻¹ for the post-2009 period were also significantly different ($H = 53.39$, $P < 0.0001$). There was no significant difference in annual dbh increment for the non-planted trees between the control and post-cultivation blocks ($H = 1.80$, $P = 0.18$); similarly the difference in annual dbh increments of non-planted trees between trees under fire protection and annual burning was not significant ($H = 0.72$, $P = 0.40$). However, planted trees under fire protection grew at a higher rate of 0.31±0.05 cm yr⁻¹ than that of 0.14±0.05 cm yr⁻¹ under annual burning ($H = 3.78$, $P = 0.05$; Figure 4b).

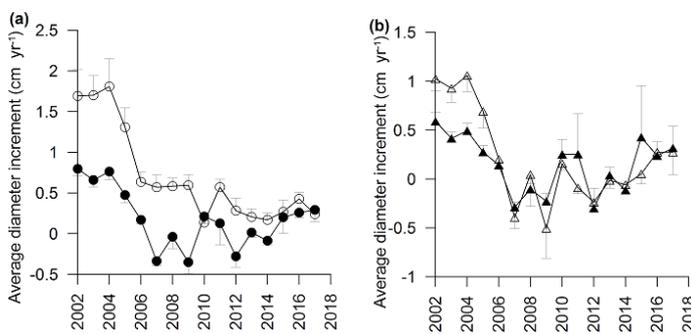


Figure 4: Trends in *Senegalia polyacantha* tree diameter increment in (a) planted (filled circles) and non-planted (empty circles) trees and (b) planted trees under fire protection (empty triangles) and annual burning (filled triangles). Vertical lines show standard error of mean and are shown in one direction for clarity.

As expected, the most important predictor of tree dbh increment was year (i.e., time after recruitment) for both non-planted ($y = 213.47 - 0.106x$, $r^2 = 0.42$, $P < 0.0001$) and planted ($y = 106.42 - 0.053x$, $r^2 = 0.33$, $P < 0.0001$) trees. For planted trees the addition of tree density to year in a two-variable model increased the explanatory power of the model by eight percent to 41% but this procedure had no effect on the single variable model for non-planted trees.

4. DISCUSSION

4.1 Seedling emergence and survival

Studies of seed dormancy in dry tropical forest trees has been limited to the seedling phase with most of these showing that mechanical scarification is the most effective method of increasing seed germination rates [20]. The findings of this study on *S. polyacantha* confirm these observations but I am not aware of studies dealing with the post-seedling effects of seed scarification. The effects of seed scarification in *S. polyacantha* on seed germination and seedlings were both positive and negative. Scarification increased the speed and rate of seedling emergence in *S. polyacantha*. The fact that a very low proportion of untreated seeds germinated after the initial wave of germination during the first season and in the second wet season indicates low levels of seed dormancy in this species and scarification appears to increase the probability of germination of seeds that would have naturally failed to germinate. The significantly higher levels of mortality among seedlings from scarified seeds than those from untreated seeds suggests that seeds that are selected against germination under natural conditions are induced to germinate but probably did not survive by the end of the first year. In fact the benefit gained by higher seed germination due to scarification is dwarfed by the higher survival among seedlings from untreated seeds. As a consequence of this, survivors among seedlings from untreated seeds are proportionately more than those from scarified seeds by the end of the first year. The benefits of seed scarification in *S. polyacantha* are therefore restricted to the germination stage only and the higher seedling mortality does not warrant the use of scarification.

4.2 Tree recruitment and survivorship

Tree recruitment among planted *S. polyacantha* occurred in a single event when plants were 5 – 6 years old. The proportion transitioning into the tree phase was 30% while 26% were still alive as saplings in 2017. However, tree recruitment from the non-planted population was continuous and recruitment represented a very small proportion of the non-planted sapling population (see Figure 2). These observations indicate that there are other constraints to tree recruitment in *S. polyacantha*, other than seedling mortality. It is apparent that *S. polyacantha* saplings consist of both fast and slow growers in the sense of who also pointed out that it takes 4 – 5 years for the top 20% fastest growing plants to reach the tree phase in *Vachellia* (formerly *Acacia*) *karroo* in Hluhluwe iMfolozi Park in South Africa and that slow growing saplings probably never transit to trees and are therefore doomed to die as saplings [21]. Observations in this study supports this proposition. It is also apparent that the low rate of sapling transition to the tree phase was a constraint to tree recruitment in both planted and non-planted populations.

The survival rate of *S. polyacantha* planted trees was significantly lower than that of non-planted trees to the extent that the former had a projected tree life span of less than 18 years (see Figure 2). Further, given the much lower mortality among non-planted trees, the longevity of these trees is likely to be much higher than that of planted trees. There was some indication that dead trees experienced lower growth rates in the two years preceding death which suggests that senescence was gradual. *Senegalia polyacantha* is regarded as a secondary succession species and the findings in this study confirmed this but also indicate that planted trees appear to be more short-lived than non-planted trees which should be considered when using this species in agroforestry and restoration projects [9,10,15]. In the short term *S. polyacantha* can be used for soil fertility improvement as the species is known to have symbiotic relationship with nitrogen-fixing bacteria, especially in fallows following abandonment of crop cultivation [6-8]. However, planted trees should be harvested within 10 – 12 years for fuel biomass before natural mortality drastically thins out the population and growth rates decline to very low levels [21].

4. CONCLUSION

Seed scarification in *S. polyacantha* to enhance germination should be applied with great caution because the benefit gained through higher germination is surpassed by the higher seedling mortality. Plants raised from scarified seeds were also less vigorous, grew at a lower rate and died very early in life than plants from untreated seed. If *S. polyacantha* seed scarification has to be used in agroforestry or restoration projects, planted trees should be harvested within 10 – 12 years for fuel biomass before

natural mortality drastically thins out the population and growth rates decline to very low levels.

Conflict of Interest: The author declares that I have no conflict of interest.

Geolocation information: The study plot is located at 15.467° S, 28.183° E, 1260 m altitude above sea level.

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