International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 7, No. 5, p. 30-37, 2015

INNSPUB

RESEARCH PAPER OPEN ACCESS

Seedling size and cotyledon retention have important influences on survival of defoliated seedlings of *Pterocarpus erinaceus* Poir. (African rosewood)

Hamza Issifu*, **Adam Jaleelu, Rikiatu Husseini**

Department of Forestry and Forest Resources Management, Faculty of Renewable Natural Resources, University for Development Studies Tamale, Ghana

Key words: Seedling survival, *Pterocarpus erinaceus*, cotyledon retention, seedling size.

http://dx.doi.org/10.12692/ijb/7.5.30-37 Article published on November 14, 2015

Abstract

Browsing by animals is a recognized bottleneck to the establishment success of tree seedlings. *Pterocarpus erinaceus* may be particularly susceptible to browsing as a result of being a highly palatable fodder plant. The fate of browsed seedlings is often uncertain because factors that determine post-defoliation survival among tree seedlings are unclear. We conducted a plant house experiment employing a cross-factored three factor design that involved two levels each of defoliation, seedling size and cotyledon retention to test three hypotheses as follows: 1. defoliation decreases survival of *P. erinaceus* seedlings; 2. larger seedlings survive defoliation better than smaller ones; 3. retention of cotyledons enhances survival and growth of defoliated seedlings. Consistent with hypotheses 1 and 2, defoliation decreased survival of *P. erinaceus* seedlings and this adverse effect was found to exist almost entirely among small seedlings. The odds of a defoliated seedling surviving was 13.4 times lower if it was small than if it was large. Partly consistent with hypothesis 3, seedlings that retained their cotyledons had a higher number of new leaves than seedlings in which cotyledons were excised, but direct effect of cotyledon retention on seedling survival was not found. Implications of findings are discussed.

* **Corresponding Author:** Hamza Issifu ✉hamza.issifu@gmail.com

Introduction

Pterocarpus erinaceus Poir., family *Fabaceae*, subfamily *Faboideae,* is a savanna tree species with enormous socio-economic benefits in its native range and beyond (Orwa *et al*., 2009; Abdul-Rahman *et al*., 2008).The species produces fine-grained timber for various uses (Duvall, 2008; Nacoulma, 2011; Abdul-Rahman *et al*., 2008; Bonkoungou, 1999) and is one of the most preferred species for charcoal production (Nacoulma, 2011; Abdul-Rahman *et al*., 2008). Also, it is an important high quality fodder species for feeding livestock (Abdul-Rahman *et al*., 2008; Duvall, 2008). However, current exploitation levels coupled with low natural regeneration of the species threatens long-term viability of wild populations (Bonkoungou, 1999). Plantation establishment of *P. erinaceus* may be the key to sustaining the benefits derived from this species.

However, browsing by livestock and wildlife is a recognized bottleneck to the establishment success of tree seedlings (Fenner and Thompson, 2005; Moles and Westoby, 2004; Asquith *et al*., 1997; Kitajima and Augspurger, 1989). As *P. erinaceus* is a highly palatable fodder plant (Orwa *et al*., 2009; Abdul-Rahman *et al*., 2008), seedlings at the nursery or those planted out in the field may suffer heavy defoliation where protective measures fail. Adventitious buds may form in response to defoliation or decapitation of plants (Barchuk *et al*., 2006) and this is necessary for seedling survival after shoot damage (Del Tredici, 2001). Nonetheless, defoliation has been reported to decrease seedling survival in many species (Kitajima, 2003; Li and Ma, 2003; Bonfil, 1998) and certain seedling characteristics such as meristematic activity at the time of disturbance as well as seedling size and age are said to influence the exact extent of defoliation effects (Bond and Midgley, 2003). Age and size of seedlings determine the amount of seedling carbohydrate reserves, and consequently, determine ability of seedlings to tolerate defoliation (Bonfil, 1998; Armstrong and Westoby, 1993).

Cotyledons function as food reserves in early plant life or as photosynthetic tissues in later stages (Kitajima, 2003). Therefore, defoliated seedlings that retain their cotyledons have higher chances of survival than when cotyledons have been removed along with all the leaves (Kitajima, 2003; Li and Ma, 2003; Bonfil, 1998). However, as seeds of *P. erinaceus* exhibit epigeal germination (Abdul-Rahman *et al*., 2008), cotyledons may be removed along with leaves by browsing animals. Smythe (1978), found that due to their high nutritional value, cotyledons are often targeted and removed by browsing animals.

Studies have been done on the effects of seed size (or seedling size) or cotyledon retention on survival and growth of defoliated seedlings for some species (see Kitajima, 2003; Li and Ma, 2003; Kitajima, 2002; Bonfil, 1998; Armstrong and Westoby, 1993; Smythe, 1978). Many of these studies have shown that defoliation reduces survival particularly of small seedlings (or seedlings from small seeds as seed size is a correlate of seedling size), and cotyledon retention have increased survival and growth of clipped/defoliated seedlings. However Kitajima, (2002), has reported differences among species that differ in patterns of seed reserves utilization as a result of having different cotyledon types. Additionally, meristematic activity at any developmental stage differs greatly among species (Paciorek *et al*., 2000). Therefore, it is important to study exact effects of seedling size and cotyledon retention for each species. Here, we present data from a plant house experiment conducted with the aim of determining exact influences of seed size and cotyledon retention on survival of defoliated seedlings of *Pterocarpus erinaceus*. This study is crucial as it helps nursery operators and plantation developers determine the fate of browsed *P. erinaceus* transplants or seedlings. Findings also serve as useful guide to seedling selection for any *P. erinaceus* planting programme.

We tested three hypotheses as follows: 1. defoliation decreases survival of *P. erinaceus* seedlings; 2. larger seedlings survive defoliation better than smaller ones; 3. retention of cotyledons enhances growth and survival of defoliated seedlings (particularly smaller seedlings).

Materials and methods

Study Site

The experiment was carried out at the plant house of the Nyankpala Campus of the University for Development Studies, Tamale. The site is located within the Guinea savanna ecological zone in the Tolon district of Northern Region of Ghana. Geographically, the district lies between latitude 9°25'N and longitude 0°58'W. Nyankpala is 16km away from Tamale, the regional capital at an altitude of 183m above sea level.

The plant house had an average mid-day temperature of 29°C in March (when the experiment was conducted). It has an apex roof which reduces irradiance amounts by up to 40%.

Seed Collection and Germination

In February 2015, we got farmers in two communities in the East Gonja district (which lies within the guinea savanna ecological zone in northern Ghana) to collect seeds of *P. erinaceus* under matured trees. In March, 2015, seeds were nursed in seed boxes after soaking in cold water for 12 hours. Seeds germinated after 7days on average. At 4 weeks, height and leaf number of all seedlings were measured. On the basis of height, seedlings were categorized into large (any seedling greater than 8cm) or small (any seedling less than 7cm) Large seedlings had an average height of 9.43cm and average height of small ones was 6.45cm.

Experimental Design

Approach employed for this study was a manipulative plant house experiment, involving three factors, each with 2 levels; seedling size (large seedlings versus small seedlings); cotyledon retention (cotyledon intact versus cotyledon excised); and defoliation (leaves intact versus leaves excised). Two controls; large seedlings with leaves and cotyledons intact and small seedlings with leaves and cotyledons intact, were included. This gave a total of 8 treatment combinations.

We employed a randomized complete block design (RCBD) so as to be able to account for the heterogeneity in light environment at the plant house. There were 7 blocks, each containing the 8 treatments. 7 seed boxes of dimensions 40cm x 10cm x 15cm were arranged on top of a concrete slab (bench) in the plant house. Seedlings were transplanted into seed boxes at a between-plant spacing of 4.5cm. The seedling size treatment was applied at the moment of transplanting seedlings into seed boxes. Transplanting was done one week prior to applying the defoliation and cotyledon excision treatment. This was to allow time for seedlings to acclimate such that observations made beyond this period would reflect treatment effects and not effects of seedling handling or transplanting. A pair of scissors was used to excise leaves and cotyledons of seedlings in the treatment group without causing any damage to the growing tips (apical meristems) of seedlings.

For the first two weeks after planting, 500ml of water per day was given to each box in a twice daily dose of 250ml morning and evening. After the first two weeks, the amount of water given was reduced to 250ml per day because soil was moist enough and evaporation appeared minimal under plant house conditions.

Data Collection and Analysis

Data on seedling survival, height and number of leaves were taken 8 weeks after the start of the experiment. Survival data was recorded as dead or alive. Absolute height growth rate was computed for each treatment by subtracting initial height (cm) from final height (cm) and dividing the result by duration of growth (in days). We computed change in leaf number per treatment to get an idea how many new leaves were produced since the application of treatments.

Associations between defoliation, seedling size and cotyledon retention with seedling survival were determined in log linear analysis, with follow up chi-square tests to separate higher order interactions.

Where data was split for chi-square analysis, expected frequencies less than 5 tended to be higher than the threshold percentage (20%) due to sample size getting smaller. As a result, we chose Fisher's exact test to compute the exact probability of the chi-square statistic, and because we had a-priori hypotheses what the effects of defoliation might be, we used the 1-sided exact significance. Odds ratio was manually computed to determine effect size of defoliation x seedling size interaction as follows:

$$Odds\ ratio = \frac{Odds\ of\ a\ small\ seedling\ surviving\ defoliation}{Odds\ of\ a\ large\ seedling\ surviving\ defoliation}$$

Where:

$$Odds\ of\ a\ large\ seedling\ surviving\ defoliation = \frac{number\ of\ defoliated\ large\ seedlings\ that\ survived}{number\ of\ defoliated\ large\ seedlings\ that\ died}$$

$$Odds\ of\ a\ small\ seedling\ surviving\ defoliation = \frac{number\ of\ defoliated\ small\ seedlings\ that\ survived}{number\ of\ defoliated\ small\ seedlings\ that\ died}$$

We performed a three way analysis of variance (ANOVA) in a GLM to assess main and interaction effects of the predictor variables on seedling absolute height growth rate and number of new leaves produced. In both analyses, "block" was specified as a random variable in the model. Comparisons were made at 0.05 level of significance. All analyses were done on SPSS.

Results

The four-way log linear analysis produced a final model that retained only lower order interactions (i.e. defoliation x survival and seedling size x survival). The likelihood ratio of this model was X^2 (10) = 8.8, p = 0.551, indicating that the model was a good fit to the data.

The results revealed significant (X^2 (1) = 16.845, p < 0.001) association between defoliation and whether or not seedlings survived, with seedling survival being lower among defoliated seedlings than the control group (Fig.1). Similarly, we found a significant (X^2 (1) = 12.596, p < 0.001) association between seedling survival and seedling size. Seedling survival was higher among large seedlings than small ones (Fig. 2). Although the log linear analysis produced a non-significant (X^2 (1) = 3.065p = 0.08) interaction effect of seedling size x defoliation on seedling survival, follow-up chi-square tests performed separately (with Yate's correction) for small and large seedlings revealed significant (X^2 (1) = 11.631, p = 0.001) adverse effect of defoliation among small seedlings, but this effect was not significant (X^2 (1) = 3.360, p = 0.111) among large seedlings (Fig. 3.).Based on the computed odds ratio, the odds of a defoliated seedling surviving was 13.4 times lower if it was small than if it was a large seedling.

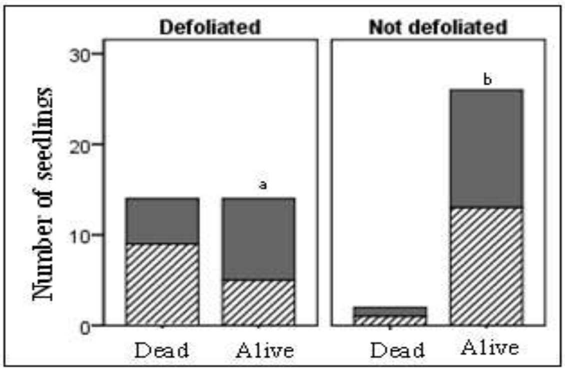

Fig. 1. Number of seedlings with cotyledons excised (Striped bars) and cotyledons intact (dark bars) separated by defoliated or non-defoliated. Comparisons made only between cotyledon excised and cotyledon intact seedlings within and across seedling size treatment. The log linear analysis showed no significant (p ≤ 0.05) interaction effect, but significant main effect of defoliation is shown with different letters.

We found no significant (X^2 (6) = 1.411, p = 0.235) association between cotyledon retention and seedling survival. The interaction of cotyledon retention with seedling size was also not found to be significant (X^2 (1) = 0.266, p = 0.606). Larger seedlings survived better, cotyledon excision regardless (Fig. 2).

Results of the ANOVA conducted on leaf number revealed that number of new leaves produced was significantly ($F_{1,3}$ = 9.941; p = 0.051) affected by the interaction of defoliation with cotyledon retention. Defoliated seedlings that retained their cotyledons produced more leaves than those with excised cotyledons, but this effect of cotyledon retention was not found among non-defoliated seedlings (Fig. 4).

Although this effect was found at borderline significance (p = 0.051), it represented a strong effect (η^2 = 0.768). We also found that the interaction effect of defoliation with seedling size on number of new leaves produced despite being non-significant ($F_{1,1}$ = 84.049; p = 0.069), represented a very strong effect (η^2= 0.988). The interaction of defoliation with seedling size on number of new leaves produced was not found to be significant ($F_{1,3}$ = 1.0; p = 0.50).

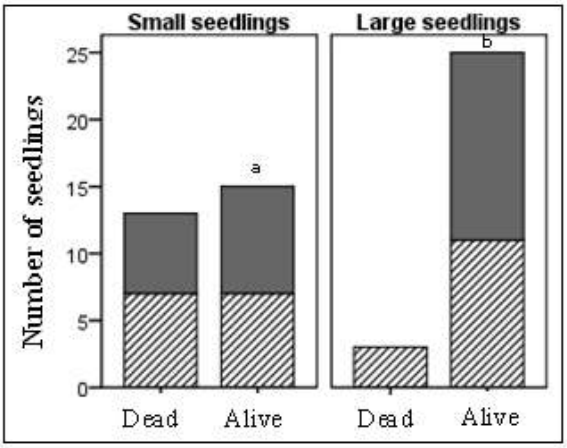

Fig. 2. Number of seedlings with cotyledons excised (Striped bars) and cotyledons intact (dark bars), separated by large or small seedlings. Comparisons made only between cotyledon excised and cotyledon intact seedlings within and across seedling size treatment. The log linear analysis showed no significant ($p \leq 0.05$) interaction effects, but significant main effect of seedling size is shown with different letters.

Although non-defoliated seedlings had higher (absolute figures) mean height growth rates than defoliated ones, particularly among large seedlings, results of the ANOVA did not produce significant differences among the various treatments (or among their interactions) (Fig. 5).

Discussion

Consistent with our first and second hypotheses, defoliation decreased survival of *P. erinaceus* seedlings and this adverse effect existed almost entirely among small seedlings. We found that the odds of a defoliated seedling surviving was 13.4 times lower if it was small than if it was large, suggesting that the ability of *P. erinaceus* seedlings to survive defoliation was dependent on size at time of disturbance. This finding lends support to findings of similar studies by Kitajima (2003), working with three Neotropical tree species; Li and Ma (2003) and Bonfil (1998), both working with oak species.This finding could be attributed to differences in amount of carbohydrate reserves between small and large seedlings. Amount of carbohydrate reserves largely determines whether or not a damaged seedling recovers, especially under shaded conditions where photosynthesis is limited (Canham, *et al.*, 1999).

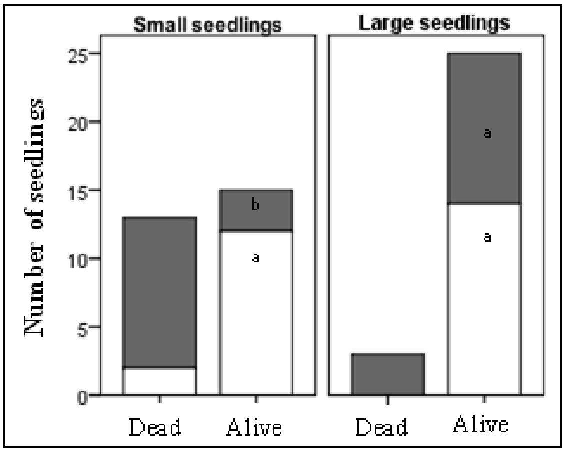

Fig. 3. Number of seedlings dead or alive of small and large seedlings that have been defoliated (black filled bars) or not defoliated (open bars). Comparisons made (Chi-square test at $p \leq 0.05$ level of significance) bbetween defoliated and non-defoliated.

Compared with small seedlings, large seedlings are known to develop proportionally large storage pith in both the stem and main root (Castro-Diez, 1998). Lower carbohydrate reserves could translate to lower resprout capacity, making small seedlings more sensitive to many disturbances, including removal of leaves (Westoby *et al.*, 1996; Leishman *et al.*, 2000). Therefore, large seedlings had the highest survival in this study because they might have depended on their stored carbohydrate reserves.

Although cotyledon retention did not have a direct effect on seedling survival, contrary to prediction, we found that number of new leaves produced by defoliated seedlings was higher among seedlings that retained their cotyledons than among seedlings without cotyledons. Cotyledons while green, contain chlorophyll and continue the process of photosynthesis to supply food to the seedlings in the absence of leaves (Raven *et al.*, 2005) and could meet the energy demands of seedlings (Kitajima, 2003). But as is common in many species with damaged areal biomass, production of new leaves was given priority in carbon allocation over height growth. Thus, none of our explanatory variables significantly affected height growth rate. Another possible reason for this finding could be that *P. erinaceus* is a slow growing species (Abdul-Rahman *et al.*, 2008). But, via its influences on leaf production, cotyledon retention could impact on seedling carbon gain and growth. Although at the end of this 8-week study such increased growth was not observed, Bonfil (1998), found this effect, because perhaps that experiment lasted longer (35 weeks).

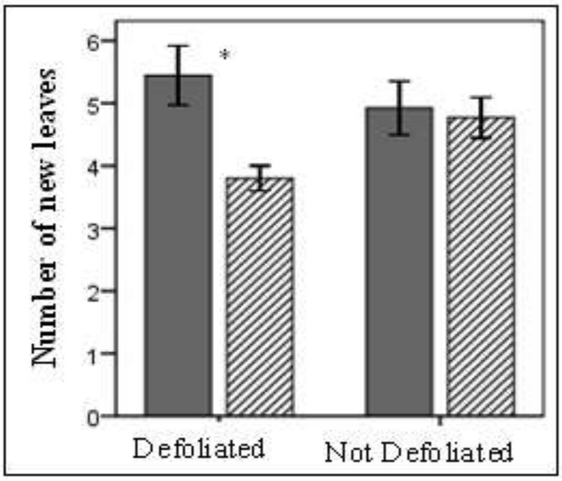

Fig. 4. Number of new leaves produced by defoliated and non-defoliated seedlings separated by whether cotyledons were excised (striped bars) or not excised (black bars). Comparisons made (ANOVA at 0.05 level of significance) produced a significant interaction effect. Significant difference indicated by asterisk. Error bars represent +/- 1 standard error of mean.

It is usually difficult to ascribe any one factor as the cause of death of a seedling (Fenner and Thompson, 2005), nonetheless, we suggest (based on our findings) that for seedlings of *P. erinaceus* to tolerate defoliation, they must be large and that tolerance may be even better if defoliated seedlings retain their cotyledons. However, as seedlings that underwent the defoliation treatment in this study only had their leaves removed and apical meristems intact, our results are valid only under the experimental manipulations we carried out (see methods). This is an important caution because exact seedling responses may depend on extent of damage to seedlings.

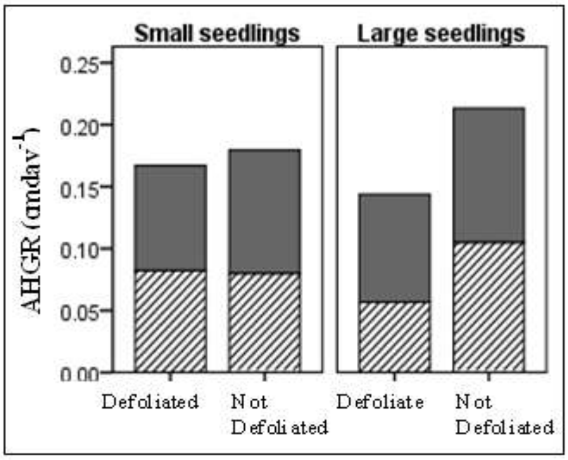

Fig. 5. Mean absolute height growth rate (AHGR) of defoliated and non-defoliated seedlings separated by whether cotyledons were excised (striped bars) or not excised (black bars). Comparisons made (ANOVA at 0.05 level of significance) produced no significant interaction effect.

Conclusion

Three hypotheses on influences of seedling size and cotyledon retention on growth and survival of defoliated seedlings of *Pterocarpus erinaceus* were tested as follows: 1. defoliation decreases seedling survival; 2. larger seedlings survive defoliation better than smaller ones; 3. retention of cotyledons enhances growth and survival of defoliated seedlings (particularly smaller seedlings). Our study revealed that defoliation reduces survival of *P. erinaceus* seedlings, but smaller seedlings suffer the adverse effect of defoliation more than larger ones. It was also shown that cotyledon retention is important for production of new leaves in defoliated seedlings. Although, direct effect of cotyledon retention on seedling growth and survival was not observed in this study, the production of more leaves by seedlings with intact cotyledons may subsequently result in more rapid growth for defoliated seedlings that retain their cotyledons than those in which cotyledons have been removed. Thus, findings in this study provide support for two of our hypotheses (i.e. hypotheses 1 and 2), but provide only partial support for hypothesis 3. We recommend that for higher tolerance of *P. erinaceus* to leaf biomass removal (and thus, higher establishment success) larger seedlings or seedlings with their cotyledons still intact should be used in any planting programme. These recommendations may be particularly useful if seedlings to be planted out are still 9cm tall or lower.

Acknowledgements

We thank senior academic staff of the Department of Forestry and Forest Resources Management for their suggestions that helped fine-tune this work.

References

Abdul Rahman AA, Ihaza OC, Oladele FA. 2008. Survey of some economic trees and their exploitation in Irepodun Local Government area of Kwara State, Nigeria. BEST Journal **6(1),** 57-60.

Armstrong DP, Westoby M. 1993. Seedlings from large seeds tolerate defoliation better: a test using phylogenetically independent contrasts. Ecology **74,** 1092–1100.
http://dx.doi.org/10.2307/1940479.

Asquith NW, Wright SJ, Clauss MJ. 1997. Does mammal community composition control recruitment in Neotropical forests? Evidence from Panama. Ecology **78,** 941-946.
http://dx.doi.org/10.1890/00129658(1997)078[0941: DMCCCR]2.0.CO;2

Barchuk AH, Campos EB, Oviedo C, Díaz MP. 2006. Supervivencia y crecimiento de plántulas de especiesleñosasdel Chaco Áridosometidas a remoción de la biomasaérea. Ecology Austral **16,** 47-61.

Bond WJ, Midgley JJ. 2003. The evolutionary ecology of sprouting. International Journal of Plant Science **164,** 103-114.

Bonfil C. 1998. The effects of seed size, cotyledon reserves, and herbivory on seedling survival and growth in *Quercus rugosa* and *Q. lausina* (Fabaceae). American Journal of Botany ***85*(1),** 79-87.

Bonkoungou E. 1999. Semi-arid lowlands of West Africa Programme, BP 320, Bamako, Mali (available at:
factnet.winrock.org/fnrm/factnet/factpub/FACTSH/P_erinaceus.html).

Canham CD, Kobe RK, Latty EF, Chazdon RL. 1999. Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves. Oecologia, **121,** 1-11.
http://dx.doi.org/10.1007/s004420050900

Castro Diez PJP, Puyravaud JH, Cornelissen C, Villar Salvadore P. 1998. Stem anatomy and relative growth rate in seedling of wide range of woody plant-species and types. Oecologia **116,** 57-66.
http://dx.doi.org/10.1007/s004420050563

Del Tredici P. 2001. Sprouting in temperate trees: a morphological and ecological review. Botany Review, **67,** 121-140.
http://dx.doi.org/10.1007/BF02858075

Duvall CS. 2008. *Pterocarpus erinaceus* Poir. In: Louppe, D., Oteng-Amoako, A.A. & Brink, M. (Editors). Prota **7(1)** Timbers/Bois d'œuvre 1. [CD-Rom]. PROTA, Wageningen, Netherlands.

Fenner M, Thompson K. 2005. The ecology of seeds. 2nd Edn., Cambridge. Book
http://dx.doi.org/10.1017/CBO9780511614101

Fenner M. 1987. Seedlings. *New Phytologist* 106 (Suppl.): 35–47.
http://dx.doi.org/10.1111/j.1469-8137.1987.tb04681.x

Kitajima K. 2002. Do shade-tolerant tropical tree seedlings depend longer on seed reserves? Functional growth analysis of three Bignoniaceae species Functional Ecology **16(4),** 433-444.
http://dx.doi.org/10.1046/j.1365-2435.2002.00641.x

Kitajima K. 2003. Impact of cotyledon and leaf removal on seedling survival in Tree species with contrasting cotyledon functions. Biotropica **33(3),** 429-434.
http://dx.doi.org/10.1646/02154

Kitajima K, Augspurger CK. 1989. Seed and seedling ecology of a monocarpic tropical tree, Tachigalia versicolor. Ecology **70,** 1102-1114.
http://dx.doi.org/10.2307/1941379

Leishman MR, Wright IJ, Moles AT, Westoby M. 2000. The evolutionary ecology of seed size. In: Fenner M (ed.), Seeds – Ecology of Regeneration in Plant Communities. CAB International, Wallingford, 31-57 P.

Li Q, Ma K. 2003. Factors affecting establishment of Quercus *liaotungensis* Koidz. Under mature mixed oak forest over-story and in shrub land. *Forest* Ecology and Management **176,** 133-146.
http://dx.doi.org/10.1016/S0378-1127(02)00274-8

Moles AT, Westoby M. 2004. What do seedlings die from and what are the implications for evolution of seed size? *Oikos,* **106,** 193-199.
http://dx.doi.org/10.1111/j.0030-1299.2004.13101.x

Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. 2009. Agroforestry tree Database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya.

Paciorek CJ, Condit R, Hubbell P, Foster RB. 2000. The demographics of resprouting in tree and shrub species of a moist tropical forest. Journal of Ecology **88,** 765-777.

Raven PH, Evert RF, Eichhorn SE. 2005. Biology of plants, 7th edition, W.H. Freeman and company Publishers, New York, ISBN 0-7167-1007-2.

Smythe N. 1978. The natural history of the Central American *agouti (Dqproctapunctata)***.** Smithson Contrib. Zoology, **157,** 1-52. UK: Cambridge University Press.

Westoby M, Leishman M, Lord J, Poorter H, Schoen JD. 1996. Comparative ecology of seed size and dispersal. Phil. Trans. R. Soc. Lond. B. **351,** 1309-1318.
http://dx.doi.org/10.1098/rstb.1996.0114

Nacoulma BMI, Traoré S, Hahn K. Thiombiano A. 2011. Impact of land use types on population structure and extent of bark and foliage harvest of *Afzelia africana* and *Pterocarpus erinaceus* in Eastern Burkina Faso. International Journal of Biodiversity and Conservation **3(3),** 62-72.