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Influence of Simulated Defoliation and N Fertilization on Compensatory Growth of *Gmelina arborea* **Seedlings**

Titus Fondo Ambebe1*, Anjah Grace Mendi2 and Abubakar Ali Shidiki3

1 Department of Forestry and Wildlife Technology, College of Technology, The University of Bamenda, Cameroon. ²

Department of Plant Biology, Faculty of Science, University of Dschang, Cameroon. ³ Department of Forestry, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Cameroon.

Authors' contributions

This work was carried out with collaboration among all authors. Author TFA designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors TFA and AGM wrote the protocol. Authors AGM and AAS managed the analysis of the study. All authors read and approved the final manuscript.

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ABSTRACT

To investigate the effect of artificial defoliation and N availability on growth of *Gmelina arborea*, seedlings were subjected to three artificial defoliation levels (0, 25, 50%) and four N regimes (unfertilized, 1 g N plant⁻¹, 3 g N plant⁻¹, 6 g N plant⁻¹) in a field trial. The results showed that height increment was 24.09% lower in the 50% defoliation than the undefoliated and 25% defoliation treatments which were not significantly different from each other. On average, the 25% and 50% defoliation treatments reduced stem volume increment by 44.34%. Increments of diameter and biomass and leaf production were not reduced by defoliation. In terms of response to N, increments in height and stem volume rose from 8.98 cm and 8.23 mm at unfertilized to 11.39 cm and 12.13 mm at 3 g N plant⁻¹, respectively, while number of new leaves increased by a margin of 1.51 from unfertilized to 6 g N plant⁻¹. Total biomass increment that was unaffected by defoliation showed an

increasing trend from 0.55 g at unfertilized and 1 g N to 0.83 g at 3 g N and 0.94 g at 6 g N plant⁻¹. There was no significant interactive effect of treatments on any parameter, suggesting that the adverse effect of defoliation on growth of *G. arborea* seedlings may not be alleviated by N fertilization. It is encouraged that a similar study be conducted for a longer duration to ascertain if the responses are sustained or modified.

Keywords: Deciduous tree; defoliation; Gmelina; growth; montane forests; N nutrition.

1. INTRODUCTION

Also known by the common name white teak, *Gmelina* (*Gmelina arborea* Roxb.) is a deciduous tree which attains up to 40 m in height and 140 cm in diameter, with a branchless bole of 6-9 m [1]. The species is one of the 33 that make up the genus *Gmelina* of the Verbenaceae family [2]. It thrives in climates with a mean annual temperature of 21-28°C and annual rainfall of 750-4500 mm. However, the optimum rainfall is 1800-2300 mm in areas with a dry period of 3-5 months [1]. With origins in Asia, there are largescale plantations in several tropical African countries including Senegal, Gambia, Sierra Leone, Cote d'Ivoire, Mali, Burkina Faso, Ghana, Nigeria, Cameroon and Malawi [3]. Although it inhabits a variety of soils, performance is best in deep moist soils with an ample supply of nutrients. This species that is of great economic and ecological significance is a component of the western Cameroon Highlands forest. The lightweight, stability, durability, and potential size of the wood make it ideally suitable for timber. It is used for the construction of window frames, doors, staircases, floors, panels, and musical instruments. Flowers produce abundant nectar for high-quality honey while the leaves are used as fodder for cattle [1]. In addition, the bark, flower, leaves, fruit, and roots are highly solicited for medicine [2]. The aforementioned and other services associated with the tree have initiated discussions on means of ascertaining its sustained availability.

The rapid growth of *Gmelina* has made the species a suitable candidate for reforestation programs. Like other trees of the western Cameroon Highlands, its regeneration is influenced by biotic and abiotic factors. Among the former, defoliators are very common on both seedlings and mature trees. Since the seedling stage is perhaps the most vulnerable in the life cycle of a plant [4,5], its processes are critical for understanding regeneration and making decisions for the success of the process. Leafcutting ants and other insects have been

reported to cause serious damage in nurseries and plantations [6]. The defoliation may have significant adverse effects on plant growth through a decline in carbohydrate supply from a reduced photosynthetic leaf surface area [7]. Foliage removal from insect outbreaks can result in as much as 100% defoliation in forest plants [8]. Defoliation of *Gmelina* may also result from poor management and browsing by ruminants [9].

Nutrient availability is one of the most important abiotic factors influencing regeneration. For instance, elevated soil N and P levels have been found to be beneficial to photosynthesis and growth of silver birch (*Betula pendula* Roth.) and sunflower (*Helianthus annus* L.) [10]. In an earlier study on initial growth and nutrition of guanandi (*Calophyllum brasiliense* Cambèss), Ciriello [11] reported a trend of increase in height, foliar area, stem and foliar dry mass with N addition. Similar observations have been made in seedlings of trumpet flower tree (*Tabebuia serrafolia* (Vahl) G. Nicholas) [12] and African mahogany (*Khaya senegalensis* A. juss) [13]. Going by the view that plant growth in tropical montane forests is Nlimited [14], fertilization may be expected to dramatically upregulate the regeneration of plants in the western Cameroon Highlands.

The main effects of insect defoliation [15] and N and P nutrition [16,17] on the growth of *Gmelina* seedlings have been well documented. They are not necessarily reflective of happenings in other tree species subjected to the same treatments [15]. Moreover, defoliation and nutrient availability are likely to occur concomitantly in natural environments, with their gradients expected to be accentuated by future climate change. In addition, the biotic and abiotic factors may interact to affect tree species in unpredictable ways. In *Eucalyptus nitens* (Deane and Maiden) Maiden, the effect of defoliation on stem growth is less severe on high- than lowproductivity sites [18]. This study explored the combined effects of simulated defoliation and N availability on growth of *Gmelina* seedlings.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Bamenda, northwestern Cameroon. The town is situated in the volcanic Bamenda – Banso Highlands between latitude 5.95 and longitude 10.15 at 1250 m asl. Characterized by two seasons, the rainy period runs from April to October while the dry season is from November to March. The wet season is humid and overcast and the dry season is hot and may be cloudy. Mean annual temperature is 21.5°C and precipitation is 2145 mm. With a mean temperature of 23.0°C, March is the warmest month, whereas, July and August with 20.1°C are the coldest. January and September are the driest and wettest months with 9 and 383 mm average precipitation.

2.2 Plant Material

On March 31, 2019, three-month old seedlings of *Gmelina arborea* were obtained from the Reforestation Task Force (RETAFO) nursery, Bamenda III Sub-division, and planted individually in polythene bags at the National Forestry Development Agency (ANAFOR) that is situated in Bamenda I Sub-division. The substrate was a 1:1 (v/v) mixture of sand and sawdust. The seedlings were of uniform size and normally developed with no visual sign of damage. They were irrigated immediately after planting.

2.3 Experimental Design

The experiment followed a split-plot design with defoliation as the plot and N treatments as the split-plot. There were three artificial defoliation levels (0, 25 and 50%) and four N regimes (unfertilized, 1, 3 and 6 g N plant⁻¹) in two replications. On June 1, 2019, the average height, diameter, and biomass of the seedlings were noted. Ten seedlings were then randomly assigned to each defoliation and N treatment. The N source was urea. The fertilization was then repeated at 1-month intervals. It was done by placing the fertilizer at 2 cm away from the stem to avoid burning of the seedling followed by watering. The defoliation was conducted using a pair of scissors. Three-quarter and one-half of each leaf on the seedling was removed in the 25 and 50% treatment, respectively. Defoliation treatments were applied once. No visible injury

caused by biotic or abiotic agents was observed during the experimental period. Irrigation was mainly by natural precipitation. The rainwater was, however, supplemented by watering with normal tap water when the need arose. The experiment was terminated on August 31, 2019.

The mean temperature of the months of June, July, and August, 2019 when the seedlings were exposed to treatments was 21, 20 and 20°C, respectively. The mean precipitation was 752.8, 893.7, and 754.8 mm for the said months.

2.4 Measurements

Three seedlings were randomly selected from each treatment and replication for data collection. The new leaves were counted and seedling height was determined by measuring the distance from soil level to the shoot apex. Rootcollar diameter was measured with a caliper. The root system was rinsed free of substrate and the seedling total biomass determined after ovendrying to constant weight at 65°C. Increments of height, diameter, and biomass were obtained by subtracting the values of the traits at the onset of treatments from those at the end of the trial while that of stem volume was calculated [19].

2.5 Data Analysis

All the morphological and biomass traits were analyzed by the following linear model:

$$
Y_{ijklm} = \mu + R_i + \delta_{j(i)} + D_k + RD_{ik} + \mathcal{Y}_{l(k)} + N_m + RN_{im} + DN_{km} + RD_{ikm} + \epsilon_{(ijklm)}
$$

$$
i = 1, 2; j = 1; k = 1, 2, 3; l = 1; m = 1, 2, 3, 4;
$$

where *Y* = response variable; *μ* = overall mean; *R* = replication; *δ* = restriction error on replications; $D =$ fixed effect of defoliation; $Y =$ restriction error on defoliation treatments; *N* = fixed effect of nitrogen treatment; ε = experimental error.

The data were checked for normality and homogeneity using probability plots and scatter plots, respectively, before being subjected to the split-plot ANOVA. A significant ANOVA result was followed by Scheffe's test for means separation. All the statistical analyses were performed using Datadesk vers. 6.1 with α = 0.05.

3. RESULTS

3.1 Height Increment

There main effects of defoliation and nitrogen supply on height increment were significant (Table 1). In contrast, the parameter did not respond to the various combinations of the treatments. The 50% defoliation suppressed height. In the case of nitrogen supply, values of height increment were lowest in the untreated

control and highest in N3. However, the differences between either N3 or the control and the two other nitrogen treatment levels were statistically insignificant (Fig. 1).

3.2 Diameter Increment

Diameter increment was unaffected by either defoliation or nitrogen supply. Similarly, no significant interactive effect of treatments was recorded for this trait (Table 1, Fig. 1).

Table 1. ANOVA p-values for the effect of defoliation (D), nitrogen supply (N), and their interaction (D × N) on growth of *Gmelina arborea*

Source			$D \times N$	
Height increment	0.0142	0.0212	0.7090	
Diameter increment	0.9894	0.7338	0.1562	
Number of new leaves	0.0592	0.0112	0.0984	
Biomass	1.0000	0.0116	0.9215	

Fig. 1. Effects of defoliation (upper-case letters) and nitrogen availability (lower-case letters) on (mean ± se) of *Gmelina arborea* **growth traits. Means underneath the same letter are not significantly different. The absence of letters above the means indicates no significant effect** *N1 = Unfertilized, N2 = 1 g N plant-1 , N2 = 3 g N plant-1 , N3 = 6 g N plant-1*

3.3 Leaf Production

There was a significant effect of nitrogen supply on numbers of new leaves. In contrast, the production of new leaves did not respond to either defoliation treatment alone or in combination with nitrogen supply (Table 1). With no significant difference between the two upper nitrogen levels, the number of new leaves increased from the control to N3 (Fig. 1). Furthermore, the difference between the control and N1 was insignificant (Fig. 1).

3.4 Biomass Increment

Biomass increment responded to nitrogen supply but not to either defoliation or interaction of the two factors (Table 1). It decreased from the N4 to N3 and finally to the control which did not differ significantly with N1 for this attribute (Fig. 1).

4. DISCUSSION

4.1 Effect of Artificial Defoliation

Although defoliation has been found to reduce height increment of forest tree seedlings, there exists a strong interaction between species and defoliation severity. For instance, while 25% defoliation induced a drastic reduction of the trait in eucalyptus (*Eucalyptus globulus*) [20], at least 75% leaf damage is required for white albizia (*Paraserianthes falcataria*) and perhaps a more severe scenario for white seraya (*Parashorea tomentella*) which did not show a response of height increment to the latter treatment [15]. In the case of red oak (*Quercus rubra*), Wright et al. [21] attributed the lack of an effect of defoliation treatments on height growth to the species' determinate growth habit and notably that growth had ceased when the treatments were applied. The findings of the present study are in agreement with the report of Chung et al. [15] that a decline in height increment of *Gmelina* is achieved at 50% defoliation.

An associated decline in stem volume increment at 50% defoliation may be explained by height rather than diameter increment since the latter was not attenuated by defoliation treatment. In contrast to our results, however, defoliation was found to reduce diameter of *Gmelina* in other studies including Chung et al. [15] and Lapis and Bautista [22]. According to Craighead [23], the influence of defoliation on diameter increment is confounded by the distance of determination up the stem axis. While we recorded diameter at the root-collar, the data presented in the other studies were collected further up the stem and this would likely have resulted in the discrepancy between our finding and that of the other investigators.

The absence of a decline in biomass following defoliation may be explained by a sustained carbohydrate supply due to photosynthetic compensation. A typical metabolic response to defoliation is an increase in photosynthetic rate of the remaining leaves. Photosynthetic upregulation has been observed in defoliated seedlings of *Q. rubra* [24] and *E. globulus* [25]. The phenomenon may be related to the exposure of shaded leaves for a greater interception of photosynthetically active radiation or an availability of greater amounts of belowground resources to the remaining leaves. Implicated in the induction of compensatory photosynthesis is an increase in leaf N, ribulose-1,5-bisphosphate carboxylase (Rubisco) carboxylase activity, electron transport or stomatal conductance [26-28]. The lack of attenuation of number of new leaves by defoliation may either alternatively or additionally have also contributed to the comparable biomass response among defoliation treatments. The fact that leaf production did not decline with defoliation is likely reflective of the species' ability to refoliate shortly after defoliation.

4.2 Effect of N Availability

There is a large body of evidence in the literature indicating that a modest increase in N is beneficial for growth. For instance, seedlings of loblolly pine showed 10, 13, and 34% increases in height, diameter, and volume in N fertilized than unfertilized treatments [29]. The addition of N to growing medium was also reported to be a requirement for maximize seedling biomass during initial nursery stages of growth, even for some leguminous plants [30]. Our results are in broad agreement with the aforementioned. The control of plant growth by N is generally due to the effect of this nutrient element on leaf growth as observed in the present study and photosynthesis [31]. N elevation increases the amounts of stromal and thylakoid proteins thereby promoting the formation of active photosynthetic pigments in leaves [28]. Furthermore, N fertilization of low-N soils augments the amount and activity of Rubisco and foliar content of ribulose-1, 5-biphosphate [32]. There exists a trade-off in allocation of N

between photosynthesis of existing leaves and development of additional leaf area [33].

The finding that the responses of height and stem volume increments and number of new leaves did not increase beyond N2 is an indication that the tissue N concentration due to this treatment was already sufficient for growth of the seedlings. According to Taiz and Zeigler [34], plants generally respond to nutrient addition with an initial growth increase up to the so called *adequate zone* where a further nutrient fertilization is reflected in an increased tissue concentration that is not translated into growth. In the *toxicity zone* that follows, any additional increase in nutrient concentration will lead to a reduction in growth. The decline in some of the growth parameters at the highest N treatment level was an expression of N toxicity as also observed in a previous study [35]. Some of the seedlings whose leaves became necrotic eventually died from the toxic tissue N levels.

4.3 Combined Effect of Defoliation and N Availability

This study was hinged on the expectation that an increase in N supply will annul the negative effect of defoliation on growth through an augmentation of photosynthetic capacity and leaf area. However, the hypothesis was not supported by our results given that there was no significant interactive effect of treatments on any parameter. In other words, the results suggest that that the adverse effect of defoliation on growth of *Gmelina* seedlings may not be alleviated by N fertilization.

5. CONCLUSION

Future shifts in temperature due to climate change can modify forest insect population dynamics. Outbreaks of potentially greater magnitude and frequency may result in massive defoliation, dieback and mortality in host plants. The phenomenon will likely be exacerbated by a decrease in the host plant resistance caused by changes in precipitation. On the other hand, an increase in atmospheric temperature is expected to positively impact N mineralization and nitrification [36]. This, together with inputs from anthropogenic sources, will lead to an overall increase in N availability in terrestrial ecosystems. The changes in these biotic and abiotic factors will likely constitute important drivers of the structure and function of the western Cameroon Highlands forests ecosystem in the future. Given that the *Gmelina* seedlings

were exposed to treatments for just a 3-month period in the present study which did not register any significant combined effect of treatments, it is encouraged that study be conducted over a longer duration to examine if the responses to the stresses are sustained or modified but also to ascertain the growth of *Gmelina arborea* seedlings in different seasonal environment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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