



Seed Pre-Treatment and Early Growth Performance of *Piliostigma thonningii* (Schum.) Milne-Redhead under Nitrogen Amendment in Old Oyo National Park, Nigeria

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Author's contribution

The author conceptualized the study, conducted it, wrote, revised and approved the final manuscript.

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ABSTRACT

Survival of wildlife in protected areas is dependent on innate attributes and response of vegetation to climate change. *Piliostigma thonningii* holds promise for wildlife conservation in Old Oyo National Park, but its population is threatened by physical dormancy. There is dearth of information on long-term benefits of its seed pretreatment and early growth response to nitrogen fertilizer. This study assessed effects of acid scarification on germination and early growth of *P. thonningii* under different nitrogen application rates. Seeds harvested from three purposively selected seasonal wetlands in Old Oyo National Park (OONP) were treated with 1N H₂SO₄ at durations ranging from 0 – 120 minutes in three replicates. Germinated seeds were enumerated over a 14-day period. Top soils from the wetlands were randomly sampled using soil auger in three replicates for physical and chemical analyses, and for early growth study with Urea fertilizer at rates 0, 50 and 100 kg/ha in

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experimental pots in completely randomized design. Growth and dry matter were assessed using ANOVA at $P.05$. Germination commenced in control treatments at 14 days after plating (DAP) and was low (13.3%). Germination progressively increased in acid treatment. At 5 DAP for soaking for 30 minutes gave 46.67%; while it was 96.67% at 40 minutes at 14 DAP. Higher soaking times gave 100% germination by 14 DAP, but with low growth vigor. The response of *P. thonningii* to Urea application was significantly higher in number of leaves at 12 weeks than in other rates. Other growth parameters are not significantly different at all rates. The biomass yield of *P. thonningii* was 1.98 g \pm 0.21 and 2.6 g \pm 0.36 root and shoot dry weights at 6 weeks after sowing (WAS); and 7.3 g \pm 0.04 root and 6.4 g \pm 0.95 shoot dry weights at 12 WAS. *Piliostigma thonningii* can restore and improve wildlife abundance to mitigate climate change effects in the Park.

Keywords: Seed dormancy; climate change; *Piliostigma thonningii*; Old Oyo National Park; urea fertilizer; early seedling establishment.

1. INTRODUCTION

The conservation of wild ungulates is intimately linked to the availability of browse and forage species in their habitats [1,2]. In the case of pronghorn, a loss of high-quality habitat and connectivity due to projected climate change was reported [2], and which may negatively influence their populations and wellness throughout their native range. Browse and forage species supply nutrients and ensure the well-being of ruminants for survival and productivity. Plants in the wild rely on the interaction of environmental elements and their gradients to survive. The most important include climatic and edaphic conditions, as well as occasional anthropogenic interventions such as bush clearing using fire, chemicals, or other physical techniques. Climate in many parts of the world is fast changing, especially in tropical areas, producing causing changes in vegetation [3].

Climate influences the eventual replacement of browsing plants with grasses that are well adapted to dry situations. This is typically exacerbated in already-dry savanna habitats. Fire and browsing by animals have an impact on browse species reporting in the wild, particularly in forests [4]. This is an example of circularity, but it must be addressed in order to establish more browse species, particularly in tropical forested savannas. This could be accomplished through enrichment planting [5], in conjunction with addressing the basic environmental and biotic reasons of species erosion.

Plants require minerals such as nitrogen, phosphorus, and potassium for growth and development. Soil fertility gradients are connected with major variations in plant species composition and plant life forms [6]. Many wetland ecologists have explored the effect of

are associated with gradients in soil fertility [6]. Soil fertility in determines vegetation composition. Verhoeven et al. discovered that various plants have been studied by many wetland ecologists. Through their experiment on biomass response to fertilization [7] some limiting factors affect plants in the seven habitat types employed in their experiment on biomass response to fertilization. They discovered that nitrogen and potassium limit plant performance twice and three times, respectively, and that N+K limits plant performance four times in wet grassland. Phosphorus limited it three times on moist heathland, whereas N and K had little effect. However, fens and dunes were found to be susceptible to nitrogen or phosphorus limitation.

An almost even split between nitrogen-limited and phosphorus-limited locations was reported in a survey of 45 studies on fertilization in seven types of herbaceous mires [8]. Similarly, that plants indicate an almost even split between nitrogen-split sites and phosphorus-limited sites [8,9]. Substrate gradients using wetland plants produced N levels in sand ranged from 0.3 g/kg to 5.2 g/kg in silty clay, while P levels ranged from 0.05 g/kg to 1.65 g/kg in sand to silty clay [9]. An extended implication of plant nutrient limitation has a long-term impact on animal nutritional shortage since plants are a primary source of these nutrients for animals. This indicates that the availability of nutrients in wetlands influences the nutritional value of wetland plants as well as animal health and residency.

Animals may be able to harvest a food source that is about 5% nitrogen dry weight (White, 1983), implying [10]; therefore, suggesting that nitrogen, not energy, is the limiting resource for animal communities [6,10]. It is important to note,

however, that while biomass and plant species composition may change with soil fertility, the per gram availability of nitrogen to animals may not because there is no difference in N concentration between plants typical of habitats with high and low fertility habitats [6,11].

Thus, the fate of many wild ungulates is dependent on the ability of protected areas to provide not only refuge but also adequate food resources. The ability of these protected areas to perform this ecological function is dependent on good management.

As one of Nigeria's eight national parks, Old Oyo National Park (OONP) is home to a diverse range of wild animal species that are nourished throughout the year by pockets of wetland vegetation provided by the wetlands around Rivers Ogun and its tributaries that through the Park. The environmental importance of Old Oyo National Park was proved by its designation as a National Park in 1988, despite the fact that it was gazetted on March 10, 1973. In the face of widespread extinction of plant species in Africa. The first step in conservation is to identify endangered species that are at risk [12]. In the instance of OONP, *Piliostigma thonningii* has been recognized as a major browse species [Olubode, 2007 unpublished thesis, 13,14].

The temporal dynamics of species are dependent on the relationship between the scale of environmental change and the time scale of organismal response [15]. Therefore, a fast growing, highly productive plant would be desirable for wildlife conservation in protected regions. Four savanna types are outlined based on meteorological and conventional zonation as Transition savanna, Guinea savanna, Sudan savanna, and Sahel short grassland. Old Oyo National Park is located in Nigeria's southern Guinea Savanna [16,17]. The savanna is described as having over 200 wet days, a precipitation/evapotranspiration ratio of 0.75 to 1.0, being burned annually, being severely disturbed by man, and typically consisting of a mosaic of agricultural land, forest remnants, and grassland.

The herbaceous components of the Park include, *Andropogon tectorum* and *Andropogon gayanus* which are richer in proximate contents than *Hyparrhenia involucreta* and *Hyparrhenia rufa* (Olubode et al., 2009) [14]. Being located in the Guinea savanna, it is characterized with a length of rainy days of 150 to 200 days.

Precipitation/evapotranspiration ratio of 0.40 to 0.75, and as being burnt annually [14,17]. *Piliostigma thonningii*, *Detarium microcarpum*, *Terminalia spp.*, *Combretum spp.*, *Burkea africana*, *Vitellaria paradoxa*, *Azelia africana*, *Daniellia oliveri*, and *Isobelinia spp* are abundant in OONP [12, 14].

P. thonningii, a well-known forage species was fast disappearing from OONP [Olubode, 2007 unpublished thesis, 14]. As an important browse plant, its fresh leaves contain high amounts of percent crude protein, crude fiber and extractable ether [18]. It is used in Zimbabwe as a supplement in cattle feed because of its high nutritional value [19]. It is suspected that prolonged dormancy offered by hard seed coat contributes to unavailability of on *P. thonningii* one hand and the depletion of nitrogen content in soil by annual burning affects the early growth of few germinated ones on the other hand (Olubode, 2007 unpublished thesis). Thus, only few mature individuals remain to replenish the Park.

Seed dormancy is a natural mechanism and an innate seed property that defines the environmental conditions in which the seed is able to germinate [20]. Plants use seed dormancy to regulate regulates its population so as to avoid unfavorable biotic and abiotic environmental conditions, or to ensure continued existence of its kind without wasting its parenting materials. It is beneficial in legume domestication and in the wild [21,22] where environmental conditions can be has and variable. Dormancy may be due to impermeability of seed coat to water and gases caused by deposition of suberin, lignin and cutin in the responsible membrane or across the micropylar opening as in most legumes; hinderance of seedling growth as a result of mechanical resistance by seed coats; embryo or physiological dormancy caused by physiological immaturity of embryo as in most grasses; the presence of growth inhibiting chemicals [22,23,24]. Seed dormancy, because it spreads seedling recruitment over a period of time in an area, is termed "temporal dispersal" [25].

Seeds of *Piliostigma thonningii* responded to heat treatment of 20°C up to 200°C for 5 minutes in an experiment to simulate heat shock caused by savanna bush burning on seeds found in upper soil layer [26]. It is reported that short exposure of seeds to high temperatures generally stimulated germination whereas

prolonged exposure reduced seed germination [26]. Pretreatment of seeds of *Piliostigma thonningii* with tetraoxosulphate VI acid gave good germination at 30 minutes – 120 minutes acid-soaking periods than when the seeds were sown immediately [27]. However, the impact of breaking the dormancy with the acid on rate of successful establishment of seedlings has not been documented. Therefore, this study investigated the germination efficiency of breaking hard seed coat dormancy in *P. thonningii* with 1N H₂SO₄ and subsequent early growth biology under nitrogen fertilization.

2. MATERIALS AND METHODS

Studies on germination and early growth biology of *Piliostigma thonningii* (Schum.) Milne-Redhead.

2.1 Germination Study

In order to have a wide pool of genotypes, *P. thonningii* fruits (pods) were collected from mature trees in the three sites and in Ibadan. The seeds were then taken out and carefully combined in a bag before being evaluated. The following pre-germination treatments were used to thaw the hard seed coat dormancy of the seeds:

2.1.1 Pre-germination treatments

Germination studies were conducted with the aim of removing the mechanical hindrance posed by hard seed coat using 1N H₂SO₄ (acid scarification). *P. thonningii* was selected by virtues of its importance as wildlife feed and irregularity of occurrence in the three wetlands. The acid scarification treatment of the seeds of the forage legume, *Piliostigma thonningii* was tried for 0, 2, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 minutes with distilled water applied for the “0” control. 10 seeds were put in a Petri dish laid with moistened 9 cm Wattman’s No 1 filter paper. The treatment were replicated thrice. The seeds were moistened regularly and number of seeds that germinated was recorded over a 14- period.

2.2 Early Growth Study: (Phenological Study)

Four seeds of *Piliostigma thonningii*, acid-scarified in 1N H₂SO₄ for 30 minutes and washed with distilled water were sown in thirty pots (22 cm deep with 19 cm surface diameter) which

were each filled with 3.5 kg of top soil collected from the Park. They were watered regularly to avoid water stress. The seedlings were thinned to one per pot one week after emergence.

Two weeks after sowing (WAS), 50% (0.01 g) and 100% (0.02 g) Urea fertilizer as recommended for use in tree seedling production (Equ. 1-6) were applied to each of ten pots designated for each treatment and containing one plant per pot. A set of another ten pots were designated as control (0% or 0.00 g Urea). These were only watered for the duration of the experiment (3 months) with no application of the fertilizer.

Data on the number of leaves per plant, stem diameter measured with a Venier Calliper, plant height measured with a meter rule, and leaf area/plant determined by graphical estimation, in which the margin of each leaf was traced on a standard graph sheet, and the area covered estimated, were collected every two weeks.

2.3 Physicochemical Properties of the Study Sites

Samples of top soil from three purposively selected wetlands in Old Oyo National Park were obtained with a Soil auger from the top 0-15 cm. They were collected in three replicates and bulked to obtain a composite sample per site before dividing into three coded replicates for physical and chemical analyses at the Central Laboratory of the Institute of Agricultural Research and Training (I.A.R. & T.), Moor Plantation, Ibadan, Nigeria according to the official methods of analysis described by the Association of Analytical Chemists [28]. The parameters determined included routine macro nutrients (Total Nitrogen, Available Phosphorus, Potassium, Calcium and Magnesium), Organic Carbon, pH, Exchangeable acidity, C.E.C. and textural composition (using USDA classification system).

2.4 Fertilizer Calculations

Application doses of Urea fertilizer, being a solely Nitrogen fertilizer (to amend Nitrogen deficiency in the soil) was based on recommended rate for low maintenance level (for newly planted trees) = 0.1 lb/sq ft Nitrogen

Where 1lb/sq ft = 450g/9 sq m (Equ. 1)

0.1 lb = 45 g.

If 45 g/ 9 sq m N is recommended, amount needed for 1 hectare will be

$$\frac{0.045 \text{ Kg}}{9} \times \frac{x10000 \text{ m}^2}{1} \quad (\text{Equ. 2})$$

= 50 Kg active Nitrogen/ha.

Available inorganic source of Nitrogen = Urea fertilizer.

Urea contains 45% N (45g N in 100g Urea.

1 ha furrow slice of soil weigh 2×10^6 Kg

Weight of soil per pot used = 3.5 Kg.

Amount of urea required per pot (containing 3.5 kg soil) =

$$\frac{x\text{Soil weight/pot (Kg)}}{2 \times 10^6 \text{ Kg Soil}} \times \frac{x\text{Recommended Rate}}{\text{Concentration of N}} \times 10 \quad (\text{Equ. 3})$$

However, since the amount will be too small to weigh with the available weighing Balance, it would be converted to grams by multiplying the above equation by 1000.

Thus:

$$50 \text{ Kg } \frac{\text{N}}{\text{ha}} (100\%) = \frac{3.5}{2 \times 10^6} \times \frac{50}{45} \times \frac{100}{1} \times \frac{1000}{1} \quad (\text{Equ. 4})$$

$$50 \text{ Kg N/ ha (100\%)} = \frac{3.5}{2 \times 10^6} \times \frac{50}{45} \times \frac{100}{1} \times \frac{1000}{1} \quad (\text{Equ. 5})$$

= 0.19g Urea (N)/ pot.

For 50% rate, the equation will be

$$50 \text{ Kg N/ ha (100\%)} = \frac{3.5}{2 \times 10^6} \times \frac{25}{45} \times \frac{100}{1} \times \frac{1000}{1} \quad (\text{Equ. 6})$$

= 0.09g Urea (N)/pot.

3. RESULTS AND DISCUSSION

The three study sites' soils were subjected to a soil analysis, which showed that they are quite comparable. While the soil of the wetland in Ajaku was a loam, that of the wetlands in Ibuya and Ipade-Aya belonged to the textural class of

sandy loam (Table 1). In comparison to the other two wetlands, Ipade-Aya's wetland had a higher pH level. The highest levels of phosphorus, calcium, and potassium were present in Ipade-Aya. It included basic salts and had the highest Cation Exchange Capacity (CEC). However, compared to the other two wetlands, it had the least organic carbon and nitrogen. The Ajaku wetland has the lowest pH, salt, and accessible phosphorus values. It had moderate levels of total nitrogen, organic carbon, potassium, and calcium.

3.1 Germination Study– Treatment of Seeds of *Piliostigma thonningii* with 1N H₂SO₄

3.1.1 First trial

Laboratory experiments were conducted on methods of breaking suspected hard seed coat dormancy of *P. thonningii* using 1N H₂SO₄ at different soaking time regimes over a fourteen day period. There were two trials, the results of which are displayed in Fig. 1 and 2.

Germination was not recorded in the control until the fourteenth day after plating (DAP), and the germination percentage was very low (13.33%) (Fig. 1). Also, the percentage germination was initially very low for 2 and 5 minutes soaking times (MST). 50% germination was recorded for 2 MST at 14 DAP and 60% for 5 minutes at 14 DAP. 60% and 63.33% were recorded for 10 MST and 15 MST at 7 DAP and 10 DAP respectively, both of which were not statistically different at 10 DAP (P<0.05). Greater responses were progressively recorded as the soaking time increases; 76.67% for 20 MST at 14 DAP. 63.63% was recorded as early as 7 DAP for 25 MST, which also had 86.67% and 96.67% at 10 DAP and 14 DAP respectively. 46.67% germinated as early as 5 DAP for 30 MST; 96.67% of the seeds germinated at 40 MST on the 14 DAP. Remaining soaking times gave 100% germination at 14 DAP. The 110 MST produced 70.67% germination as early as 7 DAP, while 120 MST produced 50% and 93.33% germination at 5 Dap and 7 DAP respectively.

Table 1. Soil chemistry and particle size distribution (n=3 ±SE) in the rooting layers of plants in the wetlands of Ibuya river, Ipade-Aya and Ajaku river in Old Oyo National Park

| Parameter/site | Ibuya | Ipade-Aya | Ajaku |
|-----------------------------|--------------|--------------|--------------|
| pH (1N in H ₂ O) | 6.383±0.090 | 6.637±0.080 | 6.19±0.070 |
| CEC (molkg-1) | 2.83±0.346 | 3.533±0.354 | 3.153±0.146 |
| Ex_AC | 0.467±0.0082 | 0.533±0.082 | 0.4±0.000 |
| B._Sat | 83.533±1.675 | 84.66±1.654 | 82.933±5.563 |
| Org._C. (me 100g-1) | 2.11±0.123 | 1.067±0.071 | 1.563±0.104 |
| Total_N (me 100g-1) | 0.45±0.128 | 0.220±0.014 | 0.303±0.047 |
| Av._P (me 100g-1) | 28.063±1.665 | 31.813±2.893 | 8.407±1.249 |
| Ca (me 100g-1) | 0.32±0.707 | 0.397±0.011 | 0.36±0.019 |
| Mg (me 100g-1) | 0.18±0.007 | 0.167±0.027 | 0.18±0.014 |
| Na (me 100g-1) | 1.523±0.29 | 1.333±0.182 | 1.33±0.129 |
| K (me 100g-1) | 0.673±0.164 | 1.163±0.131 | 0.847±0.130 |
| Sand (%) | 79.8±1.414 | 80.467±1.654 | 59.133±4.321 |
| Silt (%) | 14.067±0.817 | 14.06±2.160 | 22.067±6.377 |
| Clay (%) | 6.133±1.633 | 5.467±0.817 | 18.8±2.450 |
| Textural Class | Loamy sand | Loamy sand | Sandy loam |

3.1.2 Second trial

In the second trial (Fig. 2), it was shown that the germination percentages of the *P. thonningii* seeds to varying soaking time regimes increases as the days after plating (DAP) increases. The control showed no response to the time progression. 10% germination was recorded for 2 MST at 7 DAP, 60% at ten DAP and 70% at 14 DAP. The 5 MST gave 13.33% germination at 5 DAP, the response being as high as 93.33% at 14 DAP. The response at 10 DAP was a little lower, but 76.67% was recorded at 10 DAP. The germination percentages were very high at higher DAP for other soaking times. 100% germination was recorded as from 30 MST for 14 DAP to the 120 MST.

However, results showed that seeds subjected to more than 30 minutes soaking-time exhibited varying degrees of the scorching effect of the acid, resulting in inability of some to successfully germinate.

3.2 Study of Early Growth Biology of *Piliostigma thonningii* in Nursery

3.2.1 Plant height

The increase in height of *P. thonningii* plants under all the treatments was initially slow for the first six weeks. The height differences among the treatments were not significant ($P < 0.05$). In the first six week period, plants subjected to 0% Urea fertilizer treatment gained a mean of 4.8 cm ±0.47, those under the 50% treatment gained 5.3 cm ±0.526 and the ones under 100% increased

by a mean of 5.8 cm ±0.565. Over the following six weeks, mean gain in height were 12.6cm ±1.14, 14.4cm ± 1.455 and 14.1cm ±1.957 for 0%, 50% and 100% urea application (Fig. 3).

3.2.2 Stem diameter

The correlation coefficient ($r=0.21$) of the Urea level against stem diameter is low and was not significant. The diameters vary from (0.21-0.30) cm for 0%, (0.21-0.31) cm for 50%, and (0.23-0.32) cm for 100% Urea applications (Fig. 4). A slight rapidity in development of the diameter was noticed in the first six weeks of the study, followed by a reduction in the rate of increase (Fig. 5). The differences among the treatments were not significant. Correlation analysis ($P < 0.05$) accounted for a low non-significant positive relationship ($r=0.21$) between the stem diameter and the Urea levels.

3.2.3 Number of leaves

A mean number of 3, 3, and 4 leaves were produced as at 2 WAS for 0%, 50% and 100% Urea levels respectively. The number of leaves steadily increased to 12, 11 and 11 for the respective treatments at 12 WAS (Fig. 5). There was hardly any difference ($P < 0.05$) in the plants response to the urea treatments. This is explained by a no correlation ($r=0.00$) at $P < 0.05$, which indicated a null effect of the urea fertilizer at the 50% and 100% levels.

3.2.4 Leaf area

The mean leaf area of plants under each treatment increased slowly for the first 6 WAS as

indicated in Fig. 5: 3.43 cm²-9.39 cm² (0) %, 3.65 cm²-7.88 cm² (50%), and 3.23 cm²-10.25 cm² (100%). The differences among the treatments were not significant. Though not significant, the rate at which control plants (0% Urea) increased

in leaf area was higher than the plants on other treatments (Fig. 6). Correlation analysis accounted for small non-significant negative relationship ($r = -0.09$) between the area of the plant leaves and the urea treatments.

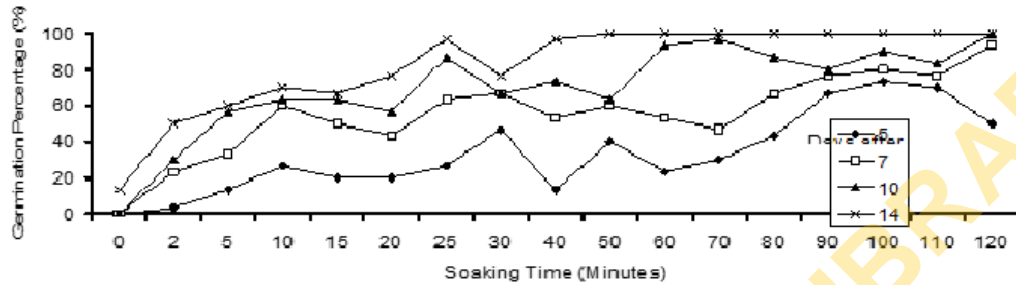


Fig. 1. Germination of seeds of *Piliostigma thonningii* at various soaking times in 1N H₂SO₄ (second trial)

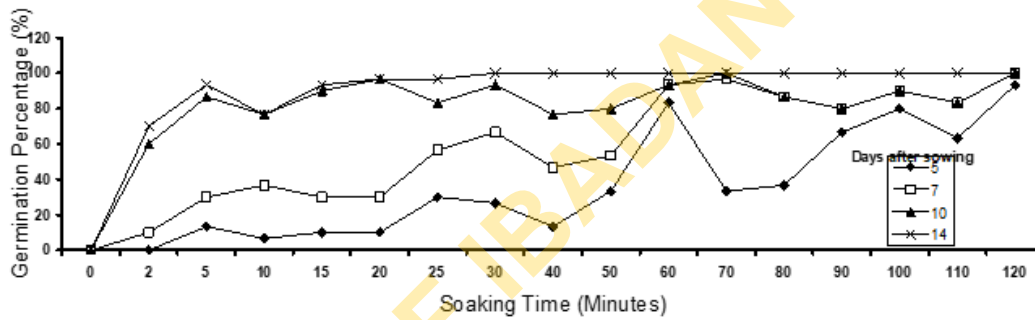


Fig. 2. Germination of seeds of *Piliostigma thonningii* at various soaking times in 1N H₂SO₄ (second trial)

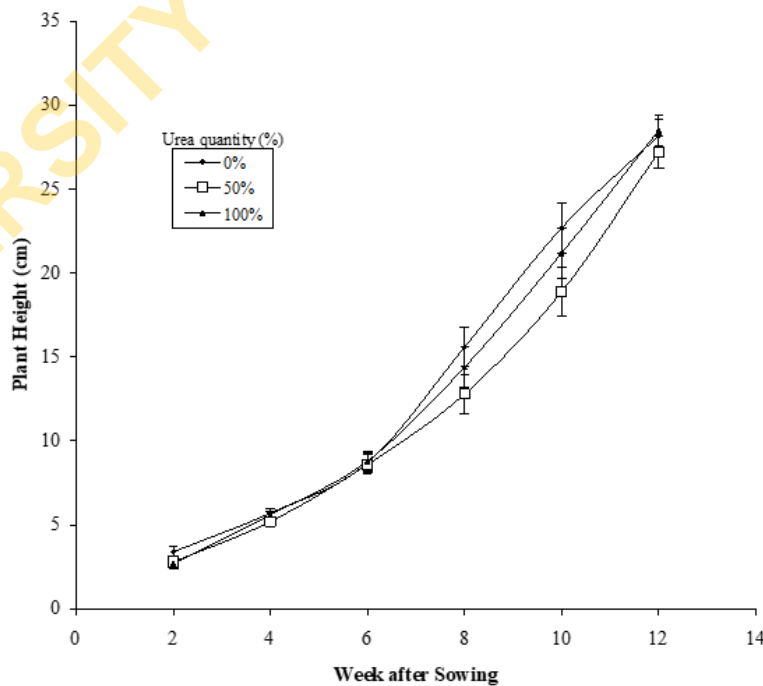


Fig. 3. Response of plant height of *Piliostigma thonningii* to different rates of urea fertilizer

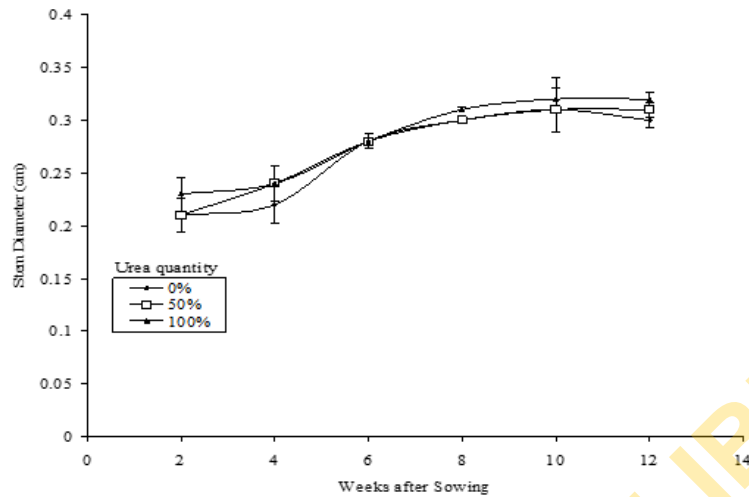


Fig. 4. Response of stem diameter of *Piliostigma thonningii* to different rates of urea fertilizer

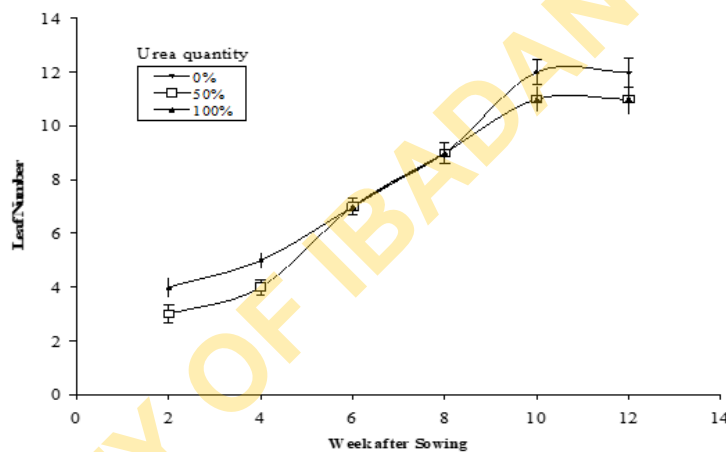


Fig. 5. Response of number of leaves of *Piliostigma thonningii* to different rates of urea fertilizer

Discussion and By creating a nursery where it could be raised until it was established enough to survive under the new fire regime before being transplanted (afforestation), the population of *P. thonningii* in the marshes could be preserved. Since the study had shown that seeds could be made to break out of their rigid seed coat dormancy when treated for 25 to 30 minutes with 1N H₂SO₄, managing the nursery would be simple. At 10 days following seeding, this treatment regimen resulted in germination rates of 83.33% to 93.33%. The nursery could be used to replant significant Park terrain where wild animals wander.

Although Ouattara and Louppe's (1992) regarding the effectiveness of breaking *Piliostigma thonningii* seed dormancy over a wide range of treatment durations (30-

120 minutes) were [27] was confirmed in this study, it was crucially discovered that treatment for shorter durations provided a more uniform performance in terms of number and growth vigor of germinated seedlings.

This study has demonstrated the ability of *Piliostigma thonningii* to repair and preserve the relationship between wildlife conservation and the availability of browsing plants in Old Oyo National Park. This finding effectively corroborates the observation of [1,2]. Thus, it can improve the health and populations of ungulates and primates [12], in the face of climate-induced degradations of vegetation in protected spaces in Nigeria, it can increase the health and populations of ungulates and primates reported for the Park [13,14], just as Raiho et al. observed [3].

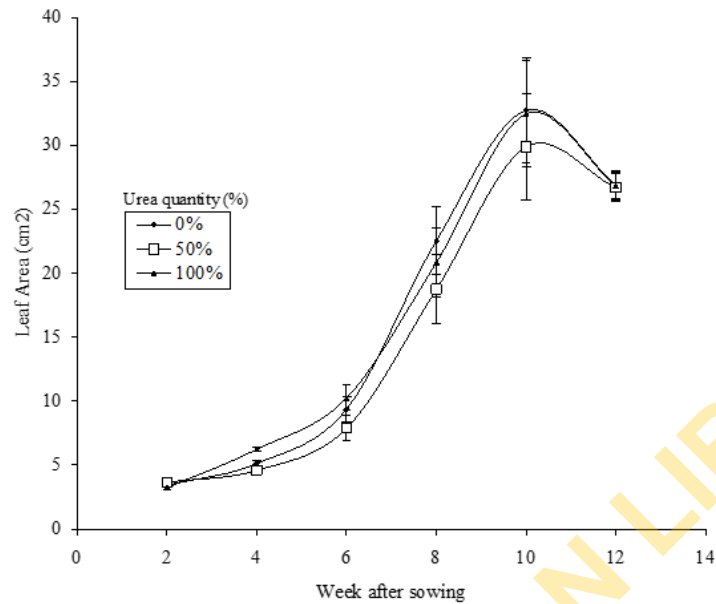


Fig. 6. Response of leaf area of *Piliostigma thonningii* to different rates of urea fertilizer

4. CONCLUSION

Although nitrogen is typically cited as a scarce resource in animal communities [6], the study found that the nitrogen-fixing *Piliostigma thonningii* could help Old Oyo National Park deal with nitrogen challenge. *Piliostigma thonningii* does not need supplemental nitrogen problem. According to the study, the shrub can function at its best during the early stages of growth without additional nitrogen.

It can be easily domesticated and multiplied in nursery without supplemental nitrogen fertilizer application or any other form of nitrogen amendment to enhance abundance and health of ungulates and primates in Old Oyo National Park, *Piliostigma thonningii* can be easily domesticated in a nursery without nitrogen fertilizer or amendment. The delicate relationship between the Park's vegetation and fauna can also be restored and maintained by using it as a planted browse species. It can further serve as a climate-mitigation shrub.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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