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Adaptability of *Moringa oleifera* Lam. (Horseradish) Tree Seedlings to Three Temperature Regimes

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ABSTRACT

Moringa oleifera trees are naturally found in tropical climates around the world, while the extent of their adaptability to cooler climates was the main objective of this study. A total of 264 trees, made up of an equal number hardened and non-hardened seedlings were randomly assigned to three temperature-controlled greenhouses each with a different fluctuating night/day temperature regime (TR) namely; 10/20˚C ± 2˚C, 15/25˚C ± 2˚C and 20/30˚C ± 2˚C. During the 32-week trial period, biweekly measurements of tree height, stem diameter and leaf area estimates of each individual tree within all three temperature regimes (TRS) were taken. The 20/30˚C TR was the most favorable towards overall tree growth, as the highest values were obtained across most measured parameters. The increase in temperature resulted in growth rate increases of over 650% between the 10/20˚C and 20/30˚C and over 250% between the 10/20˚C and 15/25˚C night/day TRS. The hardening-off pre-treatment increased both final tree height and stem diameter, resulting in increases of 3.09 × (10/20˚C), 1.44 × (15/25˚C) and 1.23 × (20/30˚C) compared to their non-hardened off counterparts. Although the average leaf area increased with the increase in TR and remained higher for the duration of the trial, cycles of regular leaf drop and renewed flushes were prevalent at both the 15/25˚C and 20/30˚C temperature treatments.

Keywords: *Hardening-Off, Biodiesel, Growth Rate*

1. Introduction

Moringa oleifera Lam. also known as Horseradish tree is one of 14 different species belonging to the family *Moringaceae*. Although indigenous to the sub-Himalayan regions in northwestern India, it is currently found in numerous countries situated in the tropical and sub-tropical regions across Africa, South East Asia and South America (Jahn, 1988) [1]. *M. oleifera* is a fast growing, small to medium sized tree ranging between 5 to 12 m in height. The tree is evergreen in tropical, while deciduous in sub-tropical climates. The tree canopy has an umbrella shaped crown with bi-(tri-)pinnate leaves, while the individual leaflets have a leaf area of one to two cm^2. Flowers are white to cream colored and zygomorphic. The tree bears 20 to 30 cm long fruit that once mature, change colour from green to brown revealing numerous round or triangular seeds with three papery wings (Folkard, *et al*., 1999) [2]. Amongst the most common uses for *M. oleifera* such as animal forage, nutrition, medicine and water purification, the seed also contains a multipurpose, non-drying oil. One of the uses for this oil is the production of biofuel, in the form of biodiesel (Rashid *et al*., 2008) [3]. Biodiesel is a renewable fuel source that is obtained through the process of transesterification where natural plant oils are transformed into a fuel, which can be used in conventional diesel engines (Poeet, 2006) [4]. Since *M. oleifera* plantations of 3270 trees/ha can yield anything between 991 - 3303 liters of oil per hectare, depending on the soil and environmental conditions, it can effortlessly compete with several other oil crops in terms of liters of oil per hectare (Fuglie, 2001) [5]. Although *M. oleifera* is grown throughout numerous African countries, no evidence of large-scale commercial plantings have been reported, possibly as a result of the limited scientific data that is currently available on both the propagation and cultivation of the tree.

2. Materials and Methods

Growth performance trials were conducted at Phytotron Section on the Experimental Farm of the University of Pretoria (25˚45′S, 28˚16′E) at an altitude of 1372 m above sea level.

Trees for the purpose of this trial were grown from seeds sourced from wild *M. oleifera* trees in Malawi. Seeds were planted and germinated in seedling trays containing Hygromix™, a sterile, soilless growing medium for seedlings, manufactured by Hygrotech Seed (Pty) Ltd. After germinating seed under favorable green-house conditions between 20˚C - 25˚C for one week, half the seedlings were left in the greenhouse, while the other half was hardened-off by placing them outside where the average minimum/maximum temperatures fluctuated between 15/30˚C. Both treatments were irrigated to field-capacity every second day. Equal numbers of hardened-off (132) and non-hardened-off (132) seedling-trees were randomly selected and transplanted into 10 liter black plastic bags, five weeks after seed was planted into seedling trays. These bags were filled with a commercial bark potting medium manufactured by Braaks (Pty) Ltd. Trees were placed onto benches inside temperature-controlled greenhouses. The abovementioned 264 trees were equally divided and randomly assigned to three different temperature regimes TRS namely, 10/20˚C ± 2˚C, 15/25˚C ± 2˚C and 20/30˚C ± 2˚C, simulating night/day temperature fluctuations and exposed to natural daylight. The average photosynthetic active radiation (PAR) at 12:00 in the afternoon on a clear day, inside the temperature-controlled greenhouses was measured at 1350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. To adjust nitrogen deficiencies evident from soil analysis results, 20 g of LAN (28) fertilizer was applied to each tree, 16 weeks after trial commencement. Irrigation was manually applied three times a week until field-capacity was reached, as the excess water was able to drain from bags.

Bi-weekly measurements of tree height (mm), measured with a measuring tape and stem diameter (mm), measured at soil level with a calliper were made of each tree for the duration of the 32-week trial.

The total leaf area estimates of each tree were calculated according to a non-destructive method of leaf area index (LAI) calculation described by Siegfried *et al.* (2007) [6]. Firstly, the number of tripinnate leaves as well as the number of leaflets on the youngest mature leaf of each tree was counted. Secondly, the number of leaflets per leaf was multiplied with the number of leaves per tree to provide an estimate for the number of leaflets per tree. Then, leaflets were randomly sampled from the three temperature treatments and measured with a LI-3100, Licor leaf area meter to provide an estimated leaf area (cm^2) of the individual leaflets. Finally the average leaf area of a single leaflet was multiplied with the estimated number of leaves per tree to provide an approximate leaf area per tree.

Data collected over the 32-week trial period were statistically analyzed using the Statistical Analysis System (SAS Version 9.1) program for Microsoft Windows, by the Statistics Department at the University of Pretoria. The Analysis of Variance (ANOVA) was performed, together with F-test (Steele and Torrie, 1980) [7] to enable the comparison between treatment means.

3. Results and Discussion

The difference in average tree height and stem diameter of both hardened-off (HO) and non-hardened-off (NHO) trees within each temperature regime (TR) for the 32-week trial duration is illustrated in **Figure 1**. Amongst the three TRS investigated in this trial, the 20/30˚C TR clearly was the most favorable for tree growth, with a final average tree height of 1970 mm and stem diameter of 28.4 mm. Growth at the 15/25˚C TR was significantly less with a final average tree height and stem diameter of 1100 mm and 16.1 mm respectively. The 10/20˚C regime was certainly not conducive to *M. oleifera* growth as hardly any noticeable growth occurred throughout the 32-week period. This TR limited the average final tree height to 480 mm and stem diameter to 8.8 mm. The effect of the fertilizer application during week 16 was responsible for the sudden change in growth line gradient. It is also noticeable how fertilizer use efficiency varied between the three TRS, while the increase in growth rate was highest at the 20/30˚C regime, the 10/20˚C regime revealed only a slight increase in response to the fertilizer application (**Figure 1**).

The final growth between the HO and NHO seedlings at all three TRS for both the tree height as well as the stem diameter was not significantly different (**Figure 1**). However, the growth rates between the HO and NHO seedlings for both these parameters differed among and within the three TRS as illustrated in **Table 1**. In addition the percentage increase in growth rate, increased with a decrease in TR, exemplifying the importance of hardening off seedlings especially if seedlings are to be transplanted into a cooler climate. Regardless of the final growing climate, the HO seedlings are at an advantage and therefore the hardening-off process is highly recommended for *M. oleifera* trees.

The hardening-off process involves several complex processes that have an effect on numerous morphological and physiological mechanisms enhancing plant growth under unfavorable environmental conditions (Villar-Salvador *et al.*, 1999) [8]. Amongst numerous other plants, the effect of hardening seedlings prior to transplanting were studied on *Rosmarinus officinalis* and *Nerium oleander* by Sánchez-Blanco *et al.* (2004) [9] and

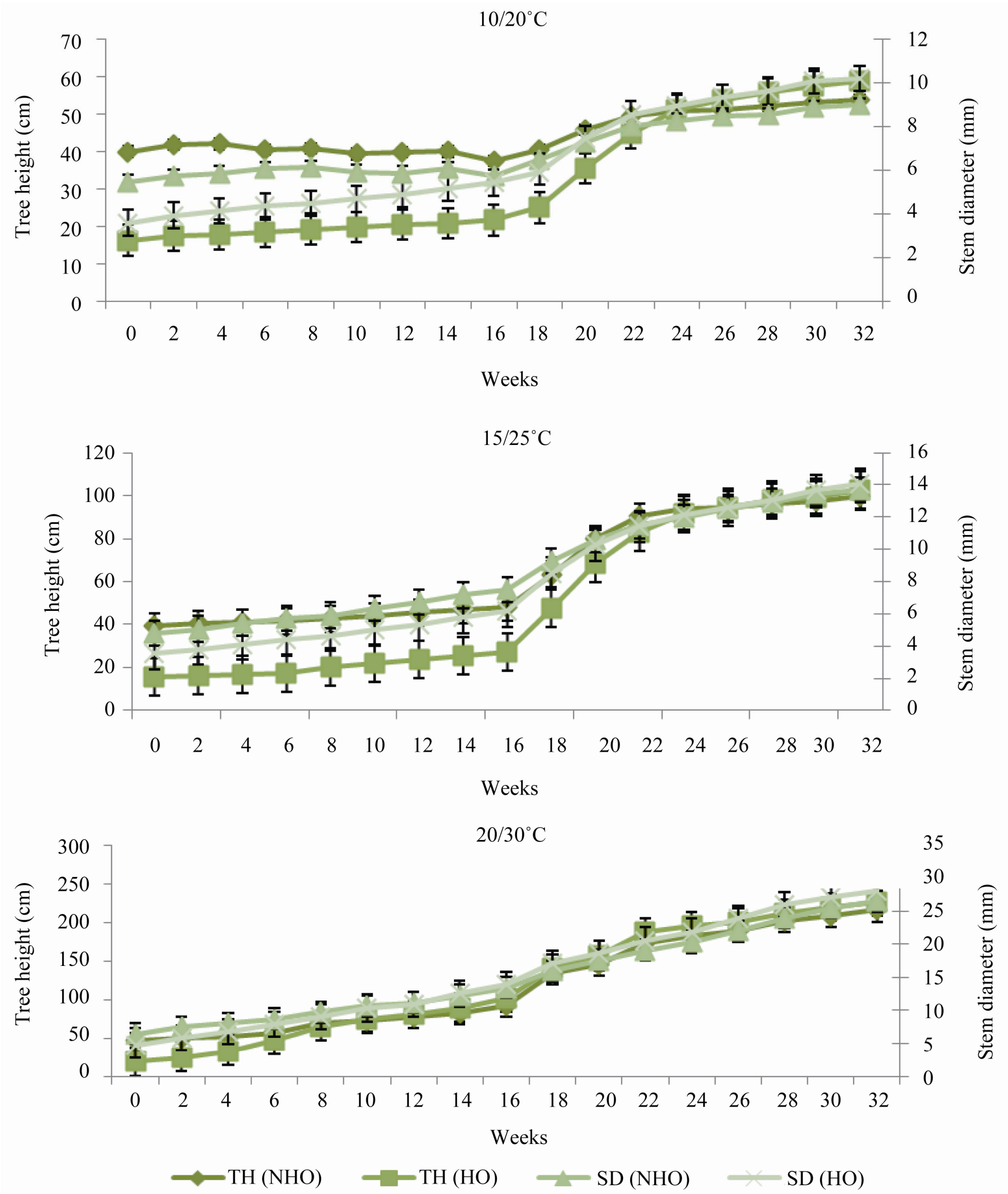

Figure 1. Differences in average tree height (cm) and stem diameter (mm) of *Moringa oleifera* cultivated from both hardened-off and non-hardened-off seedlings at various temperature regimes over a 32-week period. Vertical bars represent LSD. NHO—non-hardened-off, HO—hardened-off, TH—tree height and SD—stem diameter.

Table 1. Differences in average growth rate (mm/week) between the temperature regimes and hardening-off treatments over the 32-week trial period. Different letters indicate significant differences at $P \leq 0.05$ according to the F-test. NHO—non-hardened-off, HO—hardened-off.

Pre-treatment	Growth rate (mm/week)					
	10/20˚C		15/25˚C		20/30˚C	
	Tree height	Stem diameter	Tree height	Stem diameter	Tree height	Stem diameter
NHO	4.33^{a}	0.11^{g}	18.86^{c}	0.28^{i}	52.94^{e}	0.63^{k}
HO	13.36^{b}	0.21^{h}	27.12^{d}	0.33^{j}	65.08^{f}	0.73^{l}
Average	8.85^{ab}	0.16^{gh}	22.99^{cd}	0.30^{ij}	59.01^{m}	0.68^{n}

Bañon *et al.* (2006) [10] respectively. Both these papers reported the superiority of the hardened (HO) plants over their non-hardened (NHO) counterparts upon the exposure to unfavorable environmental growing conditions (Sánchez-Blanco *et al.* 2004 [9]; Bañon *et al.* 2006 [10]).

The growth rates between the various TRS, as shown in **Table 1**, were significantly different ($P \leq 0.05$), in which a 10˚C increase between the 10/20˚C and 20/30˚C in the night/day TR caused a tree growth rate increase of over 650% in tree height and 400% in stem diameter. While the 5˚C difference between the 10/20˚C and 15/25˚C TR resulted in a tree height and stem diameter growth rate increases of over 250% and 200% respectively. The increase in vegetative growth of *M. oleifera* trees brought about by the increase in TR agrees with similar observations made in several other tropical and subtropical trees (Menzel and Paxton, 1985 [11]; Trochoulias and Lahav, 1983 [12]; George and Nissen, 1987 [13]; Utsunomiya, 1992 [14]), such as mango (Whiley *et al.*, 1989 [15]), macadamia (Lahav and Trochoulias, 1982 [16]) and lychee (Menzel and Paxton, 1985 [11]).

According to Downs and Hellermers (1975) [17], temperature affects both physical and metabolic processes within plants, by altering the reaction rates of enzyme systems, since the optimum temperatures for enzymatic reactions are enzyme specific, and only vary between enzymatic systems. The highest growth rates are only achieved once the environmental temperature coincides with the requirements of these enzymatic reactions. As the enzymatic reactions responsible for the processing of photosynthates are temperature sensitive, growth and development are a function of the growing temperature. Temperature extremes would therefore lead to atypical and reduced growth.

The optimum TR for the purposes of this study evidently is the 20/30˚C TR, as it produced the highest growth across all measured parameters. However, according to Downs and Hellermers (1975) [17], tropical trees that reach their maximum cardinal temperature, often manifest this through rapid stem elongation, thin leaves and reduced dry matter production at the expense of reproductive development. Observations of the above symptoms under the 20/30˚C TR, suggest that any further increases in temperature would most likely decrease growth. Compared to the 20/30˚C TR, growth was significantly reduced at the 15/25˚C TR, while hardly any growth was observed at the low 10/20˚C TR. It can therefore be assumed that the threshold temperature for growth of *M. oleifera* trees is within the 10˚C - 20˚C range. This not only verifies the fact that *M. oleifera* favors tropical growing environments as stated by Morton (1991) [18] and Mughal *et al.* (1999) [19], but also demonstrates the reluctance of *M. oleifera* to acclimatize and generate satisfactory growth under cooler climates.

Similarly to the temperature effect on both tree height and stem diameter, the average leaf area (cm^2) increased with an increase in TR (**Figure 2**). However, the increase in leaf area was less steady, as fluctuations in leaf area occurred due to repeated cycles of leaf drop followed by renewed flushes. The extent of these fluctuating leaf area measurements intensified with an increase in TR and was the most volatile under the 20/30˚C regime. The 15/25˚C treatment also showed volatility, but to a lesser extent than the 20/30˚C treatment. The 10/20˚C was the most stable, showing a slight but constant increase of leaf area over the 32-week trial period.

4. Conclusions

Growth of *M. oleifera* is evidently favoured by high growing temperature of >25˚C, confirming the prefer-

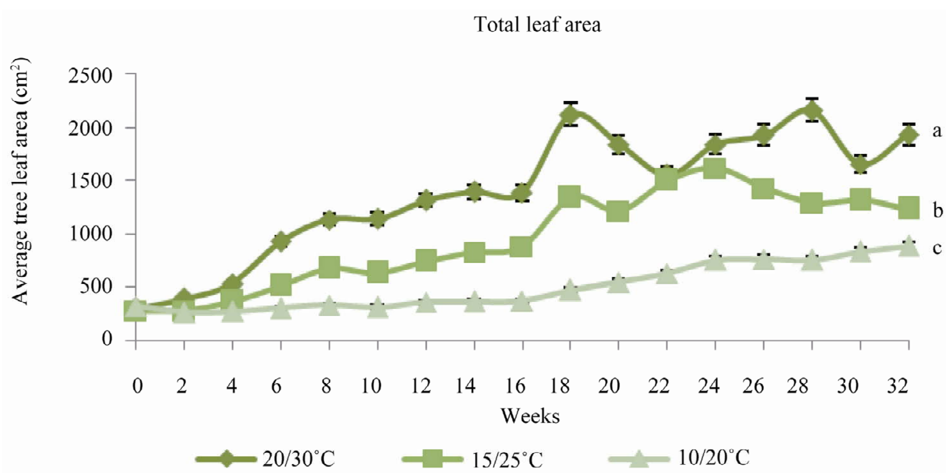

Figure 2. Increase in average tree leaf area (cm^2) of *Moringa oleifera* trees at three temperature regimes over a 32-week period. Treatment means with letters in common are not significantly different at $P \leq 0.05$. Vertical bars represent LSD.

ence of *M. oleifera* towards tropical growing environments. This was confirmed by the temperature treatment results, where trees at the 20/30˚C TR revealed the highest growth rates for both tree height and stem thickening. In addition, the 20/30˚C temperature treatment, although variable, consistently had the highest leaf area over the entire trial period. As the effect of additional, higher TRS were not investigated in this study, the possibility of improved growth at even higher temperatures cannot be excluded. Tropical climates are therefore ideal for the cultivation of *M. oleifera*, however satisfactory growth during the hot summer months in sub-tropical climates is achievable, if the winters are mild, as trees are frost tender. The hardening-off of the seedlings prior to transplanting has proven to increase the growth rate of both tree height and stem diameter across all three TRS. The hardening off process is highly recommended for *M. oleifera* trees, especially if intended cultivation is in below optimal temperature environments.

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