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SEED GERMINATION, TREE GROWTH AND FLOWERING RESPONSES OF *MORINGA OLEIFERA* LAM. (HORSERADISH TREE) TO TEMPERATURE

by

Quintin Ernst Muhl

Submitted in partial fulfilment of the requirements
for the degree MSc (Agric.) Horticulture
In the Faculty of Natural and Agricultural Sciences
University of Pretoria
PRETORIA

Supervisor: Prof. E. S. du Toit

Co-supervisor: Prof. P. J. Robbertse

May 2009

DECLARATION

I, the undersigned, hereby declare that the dissertation submitted herewith for the degree MSc (Agric.) Horticulture to the University of Pretoria, contains my own independent work and has not been submitted for any degree at any other university.

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ABSTRACT

Moringa oleifera Lam. is a tree with great potential as it is fast growing and drought tolerant. Amongst the tree's multitude of benefits, it can also be used to produce a biodiesel fuel. However, prior to the establishment of commercial plantations, all cultivation aspects of this promising tree have to be understood. Temperature is a significant climatic factor influencing both geographical plant distribution and growth, and since *M. oleifera* trees are naturally found in tropical climates around the world, the extent of their adaptability to cooler climates was the main objective of this study.

Trees were cultivated from seed and germinated in a controlled greenhouse environment at the Experimental Farm of the University of Pretoria. After germination, 50% of the seedlings were hardened-off by placing them outside under ambient growing temperatures, while the rest remained inside the greenhouse. With

trial commencement, 132 of both the hardened and non-hardened seedlings were planted into 10 ℓ plastic bags and randomly placed into three temperature-controlled greenhouses, each with a different fluctuating night/day temperature regime namely; 10/20°C ± 2°C, 15/25°C ± 2°C and 20/30°C ± 2°C. In addition, half the trees within each temperature regime were treated with the growth regulator paclobutrazol to determine its effect on growth/flowering at different temperatures. During the 224-day trial period, biweekly measurements of tree height, stem diameter and leaf area estimates of each individual tree within all three temperature regimes were taken.

Despite germination percentages being slightly higher at the low 10/20°C regime, the MGT, germination rate, uniformity and seedling growth were superior at the higher 20/30°C regime. The temperature induced seed dormancy at the 20/30°C regime, could be overcome by an incubation period at lower temperatures, as fewer instances of seed dormancy were observed at the 10/20°C regime. The increase in temperature resulted in significant ($P \leq 0.05$) growth rate increases of over 650% between the 10/20°C and 20/30°C and 250% between the 10/20°C and 15/25°C night/day temperature regimes. In addition, the 20/30°C temperature treatment, although fluctuative, consistently had the highest leaf area over the entire trial period. Hardening-off of trees during the seedling stage, significantly ($P \leq 0.05$) increased the final tree height by 3.09X, 1.44X and 1.23X, compared to their non hardened-off counterparts under the 10/20°C, 15/25°C and 20/30°C temperature regimes respectively. Leaf thickness decreased by a significant ($P \leq 0.05$) 43.1% with increase in temperature between the 10/20°C and 20/30°C regime, mostly due to a thinner mesophyll layer. The efficacy of paclobutrazol on *M. oleifera* growth was found to be temperature dependant, reducing growth at 10/20°C, while increasing growth at both

the higher 15/25°C and 20/30°C regimes. Flowering however remained unaffected by paclobutrazol. The highest instances of flowering and pollen viability were observed at the 15/25°C regime. The absence of inflorescence induction at the 20/30°C regime was responsible for the reduced flowering, signifying the necessity of vernalization prior to flowering.

Even though all the results confirm the preference of *M. oleifera* trees towards a tropical climate, satisfactory growth with possibly improved flowering during the hot summer months in certain sub-tropical climates is achievable.

Keywords: *Moringa oleifera*, temperature, paclobutrazol, hardening-off, growth, flowering

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RESEARCH OUTPUTS

The following papers (oral) were presented, based on the results obtained from this MSc study:

MUHL, Q.E., DU TOIT, E.S. & ROBBERTSE, P.J., 2008. Temperature effect on seed germination and seedling growth of a potential biodiesel tree, *Moringa oleifera* Lam. IPPS Australia – Annual Conference. Melbourne, May.

DU TOIT, E.S., MUHL, Q.E. & ROBBERTSE, P.J., 2008. *Moringa oleifera* Lam. (Horseradish tree) growth performance under three temperature regimes. Rosen Plaza Hotel, Orlando, Florida, USA. Paper presented at the 105th Annual Conference of the American Society for Horticultural Sciences (ASHS), July.

MUHL, Q.E., DU TOIT, E.S. & ROBBERTSE, P.J., 2009. Temperature effect on growth and development of *Moringa oleifera* Lam. (Horseradish tree) trees. Combined Congress 2009. Stellenbosch, January.

CONTENTS

LIST OF ABBREVIATIONS

APX	:	ascorbate peroxidase
CAT	:	catalase
cm	:	centimeter
cm^2	:	centimeter squared
CO_2	:	carbon dioxide
DAP	:	days after planting
E	:	epidermal cells
EP	:	edible portion
g	:	gram
G	:	guard cells
GA	:	Gibberellic acid
H_3BO_3	:	boric acid
HO	:	hardened-off
IU	:	International Unit
LB	:	lipid bodies
LSD	:	Least Significant Difference
m	:	meter
M	:	Mole/Molar
µm	:	micrometer
µg/ml	:	microgram per milliliter
$\mu mol.m^{-2}.s^{-1}$	:	micromole per square meter per second
mg	:	milligram
$mg.liter^{-1}$	:	milligram per liter

MGT : mean germination time

ml/m^2 : milliliter per square meter

mm : millimeter

$mmol.m^{-2}s^{-1}$: millimole per square meter per second

$mmol.s^{-1}$: millimole per second

NHO : non hardened-off

OsO_4 : aqueous osmium tetroxide

PBZ : paclobutrazol

PGR : Plant Growth Regulators

S : stomata

SD : stem diameter

SD : stomatal density

SEM : Scanning Electron Microscopy

SI : stomatal index

SOD : superoxide dismutase

TEM : Transmission Electron Microscopy

TH : tree height

TR : temperature regime

TRS : temperature regimes

TTC : triphenyl tetrazolium chloride

$W.m^{-2}$: watts per square meter

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CHAPTER 1

INTRODUCTION

1.1 Background

The world has reached an era of dwindling oil reserves and soaring oil prices, where alternative fuel sources have to be found. One such alternative fuel source is biodiesel; this 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. Although numerous oil-yielding crops are currently being cultivated worldwide for the production of biodiesel, the challenge for African countries is to find a biodiesel crop able to tolerate the harsh climatic conditions. A tree with great potential in this respect is *Moringa oleifera* Lam.

Moringa oleifera also known as Horseradish or Drumstick-tree is a fast growing, drought tolerant, medium sized tree, also able to tolerate poor soil conditions. *M. oleifera* originates from the sub-Himalayan regions of northwestern India, but is currently found throughout tropical regions worldwide. The tree is particularly renowned for its great versatility, as its uses include being a food source for humans and animals alike, coagulant for water purification, remedy for numerous ailments as well as a source for biofuel production (Anwar *et al.*, 2007). The latter is synthesized from the multipurpose, non-drying oil found inside the seeds (Rashid, *et al.*, 2008).

Based on the following quote, this dissertation seeks to provide at least partial insight into the optimal climatic conditions for the cultivation of *M. oleifera*.

“In view of its multiple uses, the *M. oleifera* plant needs to be widely cultivated in most of the areas where climatic conditions favor its optimum growth. In this way, a maximum yield of its different useable parts could be achieved to derive the maximal amount of commodities of a multifarious nature for the welfare of mankind (Anwar *et al.*, 2007).”

No significant previous research on the effect of environmental factors on *M. oleifera* has been conducted, especially outside its optimal climatic range. Amongst the various factors that determine climate, temperature is the most significant role-player affecting natural geographical plant distribution, tree performance, physiology and productivity (Sakai and Larcher, 1987; Grace, 1988). Hence the attempt to assess tree performance under three different temperature regimes (TRS) namely, 10/20°C ± 2°C, 15/25°C ± 2°C and 20/30°C ± 2°C. The latter being the temperature range where trees are naturally found at, while the two remaining regimes were chosen to determine the effect of lowered growing temperatures on trees. Temperature trials commenced by evaluating the effect of the three regimes on seed germination and seedling vigour, where after their effects were extended to tree growth under the same TRS. Prior to the placement of 264 trees into the various TRS, half of the trees within each glasshouse were hardened-off (HO) by exposing them to elevated temperature extremes, while the non-hardened (NHO) trees remained under optimal greenhouse conditions. This was done to evaluate the effect hardening-off had on the consequent growth performance. Concurrent to the tree growth trials, half the

trees (both HO and NHO) within each of the three TRS were subjected to a paclobutrazol growth regulator treatment, to quantify its effect on both tree growth and flowering. In conclusion the effect of the three TRS on flowering and pollen viability was determined.

1.2 Aim of the study

Moringa oleifera is cultivated with relative ease, consequently limited scientific trials have been conducted to expand the knowledge and improve the cultivation techniques thereof. This is endorsed by a quote taken from the book Lost Crops of Africa volume 2: “Outside certain regions of India, where large-scale cultivation is practiced, the tree receives little professional horticultural attention and has not been subjected to formal comparative trials” (National Research Council, 2006). The aim of this study was therefore to investigate the probability of *M. oleifera* cultivation in cooler climates, as they are currently only found naturally in tropical climates. Due to limited production areas in tropical climates, not only in southern Africa but also globally, the commercial plantation establishment of *M. oleifera* in cooler sub-tropical climates would significantly increase the production potential of this biofuel crop. Because of the hardiness of *M. oleifera* trees, the expansion of the production areas would also enable possible utilization of sub-optimal sites, otherwise unsuitable for the cultivation of conventional food crops.

Assessing trees under a range of TRS also enables the identification of the optimal temperature regime (TR) for those given trial conditions.

Specific objectives were

- To determine the effect of three TRS on seed germination percentage, uniformity and germination rate as well as seedling growth and development.
- To determine the growth rate and development of *M. oleifera* trees at three TRS.
- To determine the effect of hardening-off seedlings prior to transplanting on subsequent growth at three different TRS.
- To determine the response of trees to the foliar application of the growth regulator paclobutrazol at three TRS.
- To determine the effect of the three TRS on flower initiation and pollen viability.

1.3 References

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CHAPTER 2

LITERATURE STUDY

2.1 Taxonomy

Moringa oleifera Lam. is one of only 13 known species within the *Moringa* genus, which belongs to the monogerneric *Moringaceae* family. *M. oleifera* Lam. (syn. *Moringa pterygosperma* Gaertn.) is the most common, versatile and extensively utilized of all the Moringa species (Ramachandran *et al.*, 1980; Jahn, 1988). *M. oleifera* has numerous country specific vernacular names of which 'drumstick tree' (due to the shape of the fruit), 'horse radish tree' (as the roots have a similar taste to that of horse radish), or kelor tree, are the most common (Anwar and Bhanger, 2003). 'Jaffna', 'Chauakacheri Murunga', 'Chem', 'Kadu', 'Palmurungai' and 'Periyakulam 1' (PKM 1) are some of the most common *M. oleifera* cultivars. Both 'Jaffna' and 'Chauakacheri Murunga' are renowned for their sizable fruit, which are 60-90cm and 90-120cm long respectively (Tsaknis *et al.*, 1998), while 'Periyakulam 1' (PKM 1) is a dwarf variety that makes harvesting easier (National Research Council, 2006).

2.2 Distribution and habitat

Although *M. oleifera* is currently found in tropical regions throughout Africa, South East Asia and South America, its origins lie in the sub-Himalayan regions of northwestern India (Jahn, 1988). Moringa trees do not have a preference towards a

specific habitat, but do favour hot and humid environments and are thus found throughout a range of eco-zones from dry savanna to rainforests. Trees generally prefer altitudes below 600m above sea level; however in protected tropical zones they have been found to grow at elevations of up to 2000m. Because of the tuberous taproot, Moringa trees are capable of enduring anything between as little as 250mm to 1500mm of annual rainfall. The susceptibility of the trees to frost governs their natural distribution. They are able to tolerate light frost but are severely injured once temperatures drop below -5°C, even if just momentarily. Severe frost generally kills even mature trees to the roots, however, new shoots usually appear from the base of the trunk the following spring. Moringa abides with most soil types with a broad pH tolerability (from 4.5 up to 9), but preferring sandy and alluvial soils. Their vulnerability towards water logging necessitates sufficient drainage especially for clayey soils (National Research Council, 2006; Foidl *et al*., 2001).

2.3 Description

M. oleifera is a fast-growing, perennial, medium-sized tree reaching a maximum height of between 7-12m (Figure 2.1). Trees grow a straight trunk (20-40 cm in diameter) to a height of approximately 1.5 - 2m prior to the development of lateral branches. The umbrella shaped canopy, consists of numerous branch orders. Leaves are 20-70cm long, tripinnate and arranged spirally around branches. Trees also produce a tuberous taproot, rendering them drought resistant (National Research Council, 2006 ; Foidl, *et al.*, 2001).

Figure 2.1 Three-year-old *Moringa oleifera* tree growing on the Experimental Farm of the University of Pretoria.

Pinnae are imparipinnate with the terminal leaflet ob-ovate and slightly larger than the somewhat elliptic lateral leaflets that are 13–20 mm long and 3-6 mm wide. The trees remain evergreen under tropical environments, while being deciduous in temperate climates. Depending on the climate, trees either flower biannually or throughout the year, producing creamy white, pleasantly scented flowers arranged in drooping panicles 10 to 30 cm in length. The individual flowers are zygomorphic, pentamerous and 25 mm in diameter (Figure 2.2)(Ronse Decraene, *et al*., 1998).

Figure 2.2 *Moringa oleifera* flower.

Flowers are insect pollinated and produce light green, slender, three lobed, pendulous, longitudinally furrowed and angled fruit, ranging from 60 to 120 cm in length depending on the genotype. Although numerous authors refer to the Moringa fruit as pods, this term is technically incorrect, as Moringa does not belong to the Fabacae family. Once mature, fruit dry out and turn brown, splitting along the three seams revealing anything between 12 and 35 winged seeds. Seeds are round, covered by a semi-permeable three-winged seed coat (Figure 2.3)(National Research Council, 2006 ; Foidl, *et al.*, 2001).

Figure 2.3 *Moringa oleifera* seed.

2.4 Tree products and uses

Not only is *M. oleifera* the most widespread of all Moringa species, but also the most versatile, as its uses range from nutrition to medicine. Virtually the entire tree can be utilized, for example as a nutritious food for human consumption, animal forage, green manure, water purifier, medicine and even as a biofuel. Making it one of the most versatile plants, that is also of great economic value (Anwar *et al.*, 2007).

2.4.1 Food

Not only is virtually the entire tree edible, but is it also highly nutritious with above average levels of carbohydrates, protein, minerals and vitamins, making it the most nutritious amongst all tropical vegetables (National Research Council, 2006; Fuglie, 2001). The Moringa tree therefore has the added potential to fight hunger and

malnutrition, especially on the poverty-stricken African continent. A summary of the nutritional composition of *M. oleifera* leaves is given in Table 2.1, as compiled by Mughal *et al.*, (1999).

Table 2.1 Nutritional content of *M. oleifera* leaves as determined by Mughal *et al.*, (1999).

Basis of expression	***/*100g EP***
Protein	2.5g
Fat	1.7g
Carbohydrate	13.4g
Fibre	0.9g
Ca	440mg
P	70mg
Cu	1.1mg
Fe	7.0mg
Carotene	1130
Vitamin A	11300 IU
Thiamine	0.6mg
Nicotinic Acid	0.8mg
Vitamin C	220mg

*EP – edible portion

2.4.2 Medicine

Moringa oleifera is known to have numerous medicinal properties that are used to treat a range of ailments. Active compounds are found virtually throughout the entire tree, as the roots, bark, leaves, seed, oil, fruit and even the flowers are used to treat an array of ailments (Anwar *et al.*, 2007). The healing properties of *M. oleifera* include amongst others, antitumor (Makonnen, *et al.,*1997), antipyretic (Oliveira, *et al.*, 1999), antiulcer (Pal, *et al.*, 1995), antispasmodic, diuretic (Caceres *et al.*, 1992), antihypertensive (Faizi *et al.*, 1998), cholesterol lowering (Ghasi *et al.*, 2000), antioxidant (Siddhuraju and Becker, 2003), hepatoprotective, antibacterial and fungicidal activities (Ruckmani *et al.*, 1998). Additional medicinal benefits on this "miracle tree" include the treatment of cardiovascular, gastrointestinal, hematological and hepatorenal disorders (Anwar *et al.*, 2007).

2.4.3 Biofuels

In addition *M. oleifera* seeds have an oil content of between 35-40% (Rashid *et al.,* 2008). Figure 2.4 depicts a sectioned *M. oleifera* seed revealing the key constituents namely proteins, carbohydrates and oil. Following the application of various staining techniques in an attempt to identify the three main components, the lipid bodies (LB) within seeds were found to be the undefined portion in-between the larger protein and carbohydrate bodies. Amongst numerous other uses, this multipurpose non-drying oil was recently analysed and found suitable for the production of biofuel, in the form of biodiesel (Rashid *et al.,* 2008).

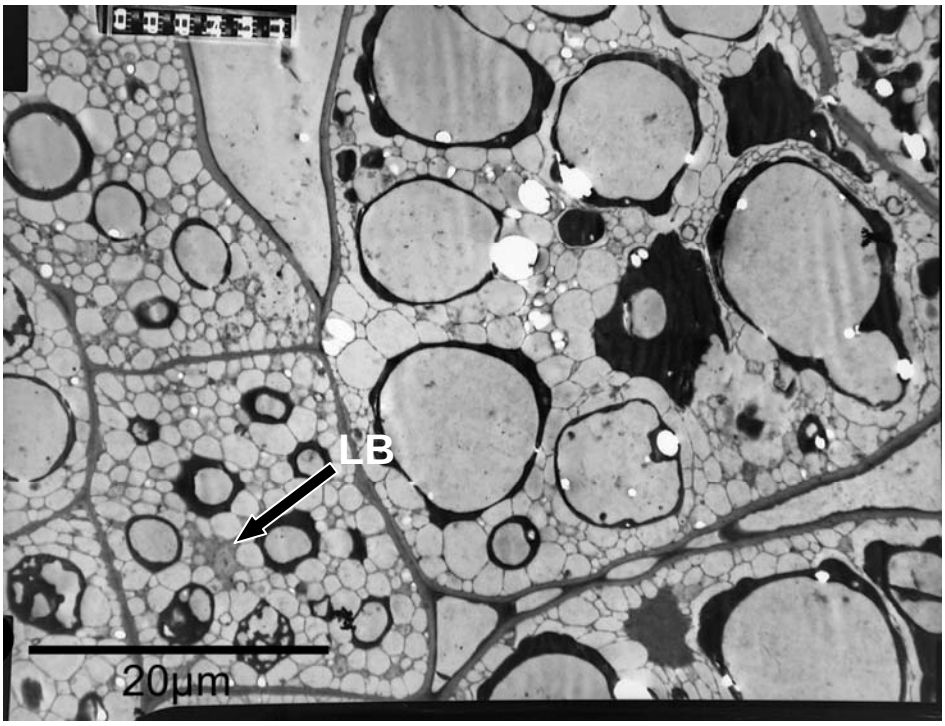

Figure 2.4 Transmission electron microscopy (TEM) section illustrating the composition of *M. oleifera* seed tissue. The intracellular lipid bodies (LB) are found in-between the larger and more prominent protein and carbohydrate bodies.

Biodiesel is a renewable fuel source produced from vegetable oils and animal fats while being an environmental friendly alternative to the conventional petroleum-based diesel fuel. The process of transesterification transforms the oil into the fuel that can be used in conventional compression-ignition petrodiesel engines. Contrary to petroleum-based diesel fuel; biodiesel is renewable, non-toxic, biodegradable and produces less harmful emissions (Knothe *et al.*, 2005).

Rising fuel prices accompanied by dwindling oil reserves have prompted the search for alternative fuel sources. Numerous crops are already being used for the production of biodiesel, such as rapeseed, sunflower, palm oil, soybean etc. across the world (Knothe *et al.*, 2005). The use of these conventional biodiesel sources have negatively affected food production, availability and prices (Scanes, 2008).

Several alternative biodiesel crops have been identified, one of which is *M. oleifera*. Rashid *et al.* (2008) have analyzed *M. oleifera* oil, and found it suitable for the production of biodiesel. During this analysis it was discovered that biodiesel produced from *M. oleifera* oil had one of the highest cetane numbers found for biodiesel namely 67.07 (Rashid *et al.*, 2008). Cetane number is a dimensionless indicator for the ignition quality of diesel fuel, the higher the cetane number the shorter the ignition delay (Knothe *et al.*, 2005). Additional fuel properties such as cold flow, kinematic viscosity, oxidative stability and lubricity were also determined by Rashid *et al.* (2008) to ascertain its suitability as a biodiesel fuel.

Prior to the establishment of commercial *M. oleifera* plantations, for the production of biodiesel, comprehension of several cultivation aspects is essential. One of the key factors influencing any plant growth, and especially *M. oleifera*, due to its prevalence in tropical climates, is temperature.

2.5 Environmental requirements

Temperature is one of the most important uncontrollable climatic factors governing natural geographical plant distribution, tree performance, physiology and productivity (Sakai and Larcher, 1987; Grace, 1988). The temperature sensitive enzymes governing metabolic pathways within plants are the first to be affected by temperature abnormalities. Photosynthesis, growth and respiration are some of the plant processes controlled by metabolic pathways; that would indirectly be affected by any temperature variation (Raghavendra, 1991).

As neither soil type nor rainfall, seem to considerably deter the growth of *M. oleifera* trees, temperature seemingly is the key factor influencing their dispersal and productivity. A temperature range of 20-30°C is considered optimal for their cultivation, with no specific upper temperature limit known to date, however, trees have been observed to survive temperatures of up to 48°C momentarily (National Research Council, 2006).

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CHAPTER 3

TEMPERATURE EFFECT ON SEED GERMINATION AND SEEDLING GROWTH OF *MORINGA OLEIFERA* LAM.

3.1 Summary

High germination percentages and rates, with relatively good uniformity, are important factors for successful commercial seedling production. *Moringa oleifera* seeds were planted into seedling trays and placed into three temperature-controlled greenhouses with fluctuating night/day temperatures regimes namely; 10/20°C ± 2°C, 15/25°C ± 2°C and 20/30°C ± 2°C. Seedling trays were monitored daily over a period of 40 days, to record differences in germination percentage, rate, uniformity and seedling growth.

Seed at the high 20/30°C temperature regime (TR) exhibited a significantly (P≤0.05) higher germination rate and uniformity compared to the two lower temperature regimes (TRS). The 74% germination percentage of the 20/30°C TR was the lowest, and differed from the 88% of the low 10/20°C TR. Viability testing of un-germinated seed revealed that although germination percentages increased with the decrease in TR, this was not as a result of uneven seed viability between samples. Temperature was thus the single factor responsible for the variation in germination percentages between the TRS.

In addition to the germination trial, the higher 20/30°C TR also favoured seedling growth, as the seedlings growth increased exponentially with an increase in temperature. From the three TRS studied during this trial, the 20/30°C TR was found to be the most favourable regime for both germination and seedling growth.

3.2 Introduction

From a commercial point of view, seed is the cheapest, easiest and most common plant propagation method. Furthermore *M. oleifera* seeds are also readily available due to the launch of seed orchard programs introduced by the Kenyan Forestry Research Institute (National Research Council, 2006). Seed germination is initiated through rapid water uptake, followed by the activation of metabolic pathways which finally lead to the first visual sign of germination namely the protrusion of the radicle (Hamley, 1932; Jann and Amen, 1977). The principal factors influencing seed germination are temperature, water, oxygen and light, of which temperature is the most significant (Hartmann *et al.*, 2002), as it affects both the germination percentage and germination rate (Edwards, 1932; Finch-Savage and Leubner-Metzger, 2006).

As no substantial information on *M. oleifera* seed germination could be found to date, the objective of this study was to investigate the temperature effects on both seed germination and seedling growth under greenhouse growing conditions. This Chapter aims to enable approximation on germination and seedling growth performance under field conditions and/or to optimize the germination under controlled nursery conditions. Since seed germination and seedling growth do not

necessarily share the same optimum temperature (Hartmann *et al.*, 2002), this Chapter deals with seed germination and seedling growth separately.

3.3 Materials and Methods

3.3.1 Seed germination and seedling growth

Seeds used in this germination and seedling growth trial were sourced from *M. oleifera* trees growing in Malawi. Seeds (Figure 2.3) were cleaned and randomly planted into three, 98-cavity seedling trays filled with Hygromix™, a sterile, soilless growing medium for seedlings containing peat and polystyrene manufactured by Hygrotech Seed (Pty) Ltd. A single 98-cavity seedling tray was then placed into each of three temperature controlled greenhouses under the following TRS namely, 10/20°C, 15/25°C and 20/30°C, simulating night/day temperature fluctuations. A margin of ± 2°C from the set temperature was permissible, due to the influence of influx radiation. Improved seed germination and seedling growth is often observed under alternating (day/night), rather than constant germination temperatures. The use of fluctuating germination temperatures is standard practice in seed-testing laboratories (ISTA, 2006), with a mandatory 10°C difference between day/night temperatures (USDA, 1952). Day length and light intensity were not controlled, as seedlings were subject to natural sunlight and the seasonal variability thereof. The temperature-controlled greenhouses used in this trial are situated at the Experimental Farm of the University of Pretoria (25°45′ S, 28°16′ E) at an altitude of 1372m above sea level. Complete weather data for the entire trial period is given in the Appendix (Table 8.A3). From the date of planting in the beginning of February, the seedling trays were monitored daily over a period of 40 days to document the

differences in germination, date of emergence, seedling height, stem width and number of leaves of each seedling among the various TRS.

Seed germination can be assessed by three parameters namely, germination-percentage, rate, and uniformity. Germination percentage is defined as the percentage of seeds from a seed population that produce a seedling, while germination rate is the speed or velocity with which the seeds germinate. Germination uniformity however, is how close in time seeds germinate (Hartmann *et al.*, 2002). To be able to quantify the effect of the different TRS on seed germination, data was transformed by calculating the mean germination time (MGT), which is the average time seeds took to germinate at each TR. The MGT is measured in days and was calculated according to the following formula from Ellis and Roberts (1980):

$$MGT = \Sigma(Dn)/\Sigma n$$

Where n is number of seeds germinated on day D, while D is number of days counted from the beginning of the germination trial. Germination curves were used to illustrate the germination test results seeing as they were found to be the best (Hartmann *et al.*, 2002)

The parameters used to assess seedling growth namely seedling height and stem diameter were measured with a measuring tape and caliper respectively. Throughout this period the seedlings received a single irrigation on a daily basis, which wetted the growing medium to field capacity while no additional fertilizer was applied.

3.3.2 Viability test

In addition to the germination trial, a seed viability test was performed to provide insight into the viability of seeds that failed to germinate at the various TRS. As a result of the partial decomposition of ungerminated seed from the seed germination and seedling growth trial, a fresh seed lot was used for the seed viability testing. A standard germination test, using the rolled towel technique was conducted according to Hartmann, *et al.* (2002). A total of 300 seeds were divided between the three TRS, by placing 100 seeds between sheets of moist paper toweling, rolled into cylinders and placed vertically into each of the three temperature controlled greenhouses. Seeds were left to germinate at the three TRS, whilst the viability of those that did not germinate was tested using the tetrazolium viability test. The test procedure was followed according to the guidelines of the International Seed Testing Association (ISTA, 2006). The already pre-moistened seed from the rolled towel germination test were cut longitudinally without damaging the embryo. A 0.1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) was prepared by dissolving 1.0 g of TTC in 100 ml of buffer solution (pH 7). Seeds were submersed in the TTC solution for 20 hours prior to their assessment.

Data collected over the 40-day 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) to enable the comparison between treatment means.

3.4 Results and Discussion

3.4.1 Seed germination

3.4.1.1 Germination test

The effect of the different TRS was initially observed on seed germination. Differences in MGT between the three TRS are illustrated in Figure 3.1.

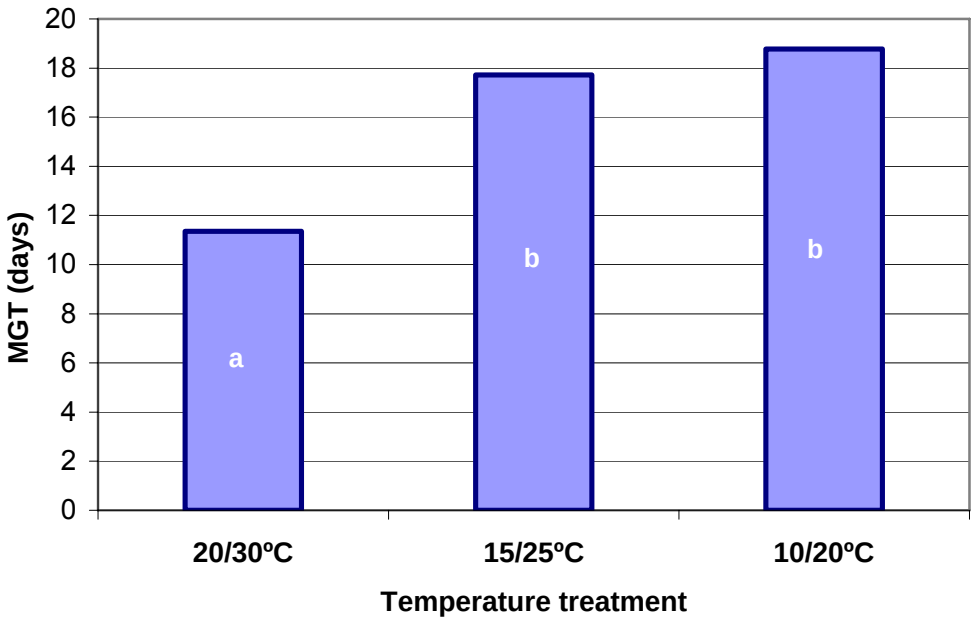

Figure 3.1 Mean germination time (MGT) of *Moringa oleifera* seed under three different temperature regimes. Treatment means with letters in common are not significantly different at $P \leq 0.05$.

Figure 3.1 illustrates the difference between the MGT of the high 20/30°C, to the moderate 15/25°C and low 10/20°C TRS. The decrease in temperature evidently increased the germination time. According to Washitani and Saeki (1986) temperature is one of the most important factors affecting the germination rate of

non-dormant seeds. In *Geranium carolinium* (Washitani, 1985), *Pinus densiflora* and numerous other plant species, the germination rate increases linearly with an increase in temperature (Washitani and Saeki, 1986), and in so doing decrease the MGT. Results from trials conducted within the Department of Plant production and Soil Science at the University of Pretoria by Mng'omba *et al.*, (2006) endorse these findings. During incubator germination trials on *M. oleifera* seed, they found the MGT to be 17 days at 25°C and only 9.4 days at 32°C. These results support the susceptibility of *M. oleifera* seed to lower temperatures as the MGT is reduced under both the lower 15/25°C and 10/20°C TRS compared to the higher 20/30°C TR.

The effects of the three TRS on germination percentage and germination rate are given in Figure 3.2. All three TRS portray the typical sigmodial germination curve. However differences were apparent in the germination rate between the 20/30°C and the other two lower TRS. Comparisons of the germination percentages among the three TRS revealed a slight reduction in germination percentage with the increase in TR. Although the lowest (10/20°C) TR had the highest overall germination percentage (93%), the differences in final germination percentage between the three TRS were non-significant.

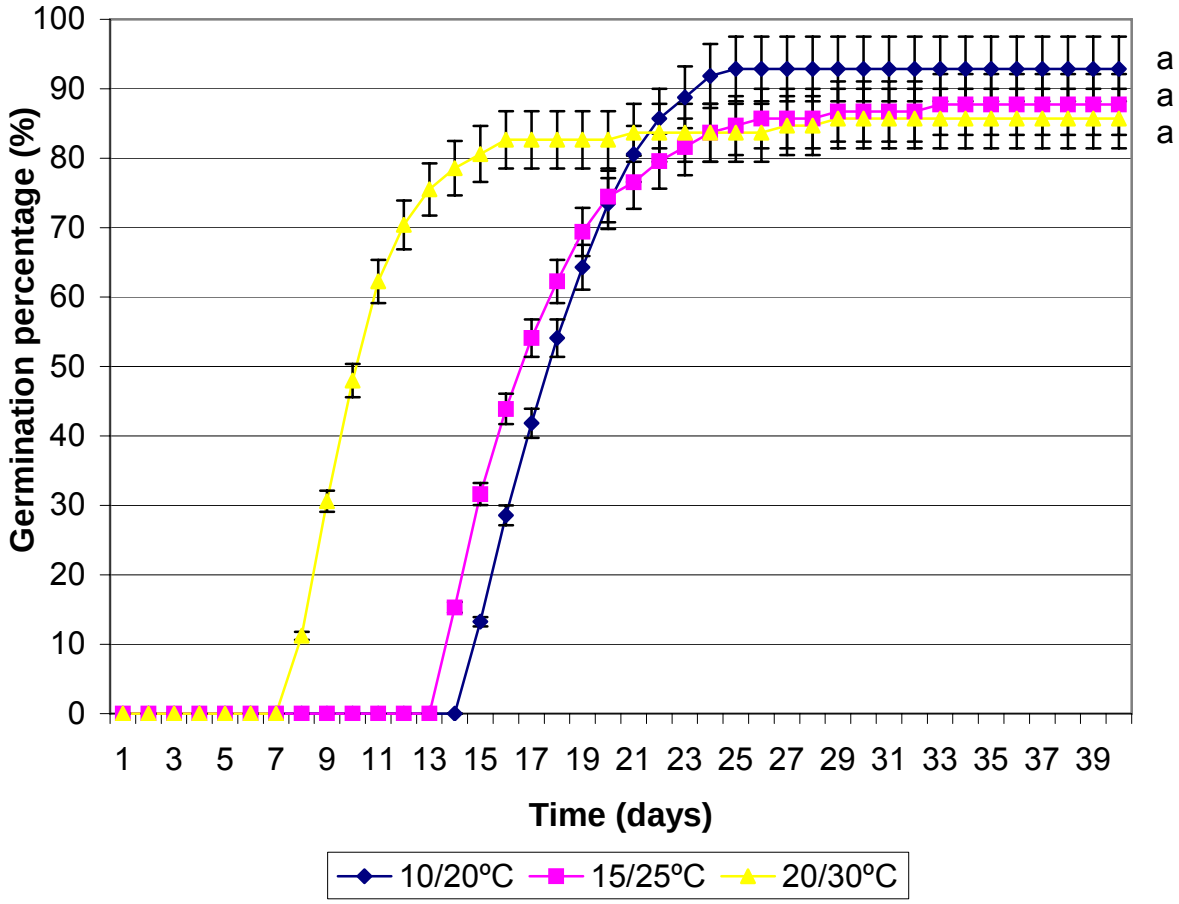

Figure 3.2 Germination percentage of *Moringa oleifera* seed under three temperature regimes over a 40-day period. Vertical bars represent LSD. Treatment means with letters in common are not significantly different at $P \leq 0.05$.

Figure 3.3 illustrates the germination curves that are representative of the germination rate and uniformity of each TR. A narrow distribution curve is indicative of a rapid germination rate and uniformity, while wider curves demonstrate poor germination uniformity. The time (days after planting) at which the curves peak, is the MGT for the particular TR. From the 20/30°C TR curve, it is thus evident that this regime had the highest germination vigour, uniformity and shortest MGT. As for both the moderate 15/25°C and low 10/20°C TRS, the curves were skewed to the right which is indicative of poor germination uniformity, while their position on the timeline demonstrates their delayed MGT. The consequence of poor germination uniformity

as observed at both the 10/20°C and 15/25°C TRS ultimately is an irregular seedling stand. However despite reduced germination uniformity at both the 10/20°C and 15/25°C TRS, they reached higher final germination percentages compared to the 20/30°C TR (Figure 3.2). For commercial seedling production prompt and uniform germination is desirable. The TR most beneficial in this respect is the 20/30°C regime, and is therefore the recommended regime for seed germination and seedling production.

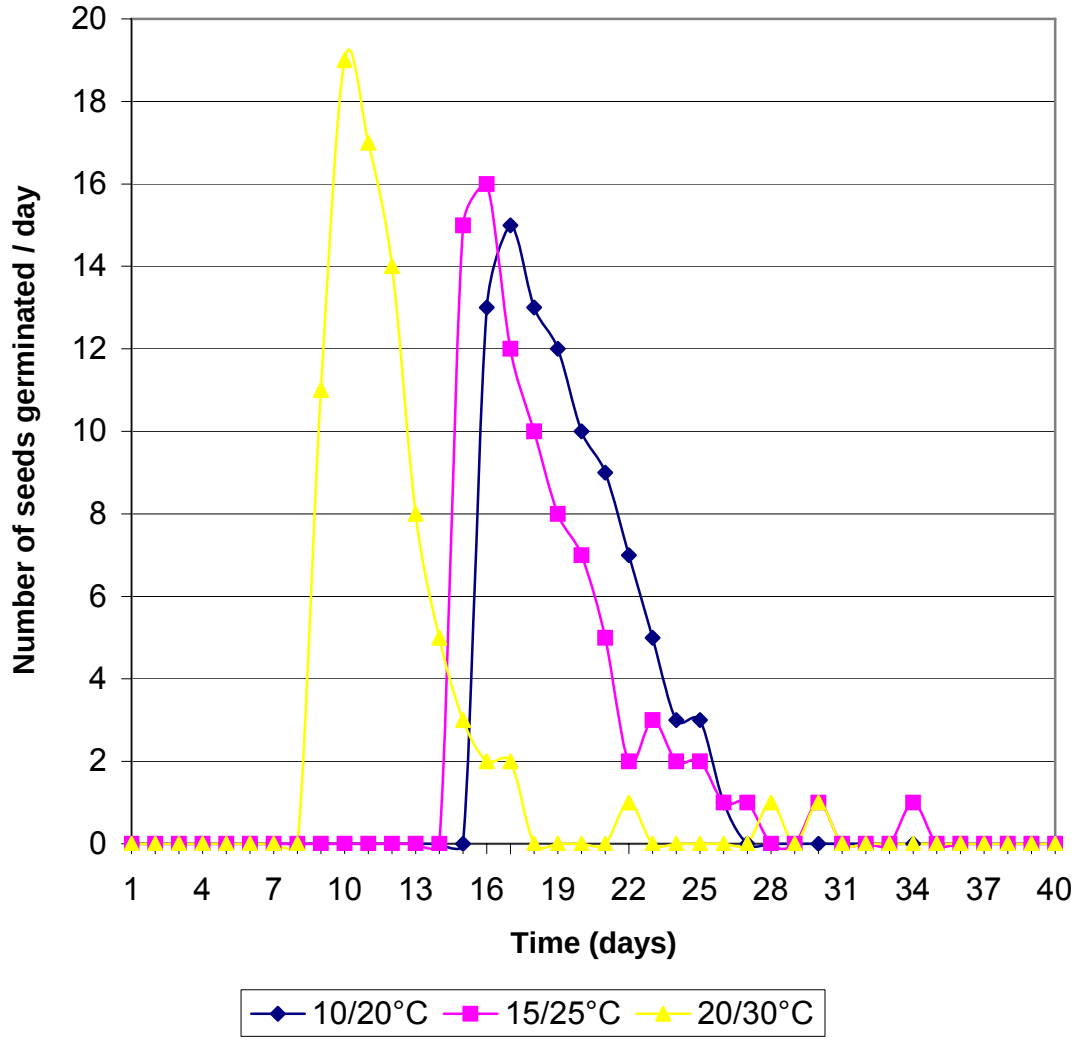

Figure 3.3 Germination curves of *Moringa oleifera* seedlings at the three temperature regimes over a 40-day period.

3.4.1.2 Viability test

Subsequent to the initial germination and seedling growth trial, a rolled towel test was conducted at the same TRS with fresh seed to gain insight into the viability of ungerminated seed. This was required due to the variation in germination percentages across the three TRS. The tendency of increased germination percentages at lower germination temperatures was observed during the greenhouse germination trials as well as by Mng'omba, *et al.*, (2006) during preliminary incubator germination trials conducted within the Department of Plant production and Soil Science at the University of Pretoria.

The tetrazolium seed viability test was performed on all seed that failed to germinate during the rolled towel germination trial. Results from the seed viability test are illustrated in Table 3.1 and Figure 3.4. Table 3.1 illustrates the germination percentages as well as the total seed viability that was determined using the tetrazolium test at the three TRS. Although differences could be found in germination percentages between the 10/20°C and 20/30°C TRS, the tetrazolium viability test revealed no differences in the total seed viability between the three TRS. This illustrates that the difference in germination percentages between the three TRS is attributable to temperature as well the presence of slight seed dormancy and not seed viability.

Table 3.1 *M. oleifera* seed viability at the three temperature regimes determined using the rolled towel germination and tetrazolium tests. Different letters indicate significant differences at $P \leq 0.05$ according to the F-test. TTC - 2,3,5-triphenyl tetrazolium chloride seed viability test.

		Temperature regime		
Number of seed		**10/20°C**	**15/25°C**	**20/30°C**
Germinated	viable	88[a]	81[ab]	74[b]
Un-germinated	viable (TTC)	4[a]	8[ab]	14[b]
Total viable seed		**92[a]**	**89[a]**	**88[a]**
Un-germinated	non-viable (TTC)	8[a]	11[a]	12[a]
Total seed		**100[a]**	**100[a]**	**100[a]**

The proportion of viable (dormant) seed amongst the ungerminated seed was determined by means of the tetrazolium test and is demonstrated in Figure 3.4.

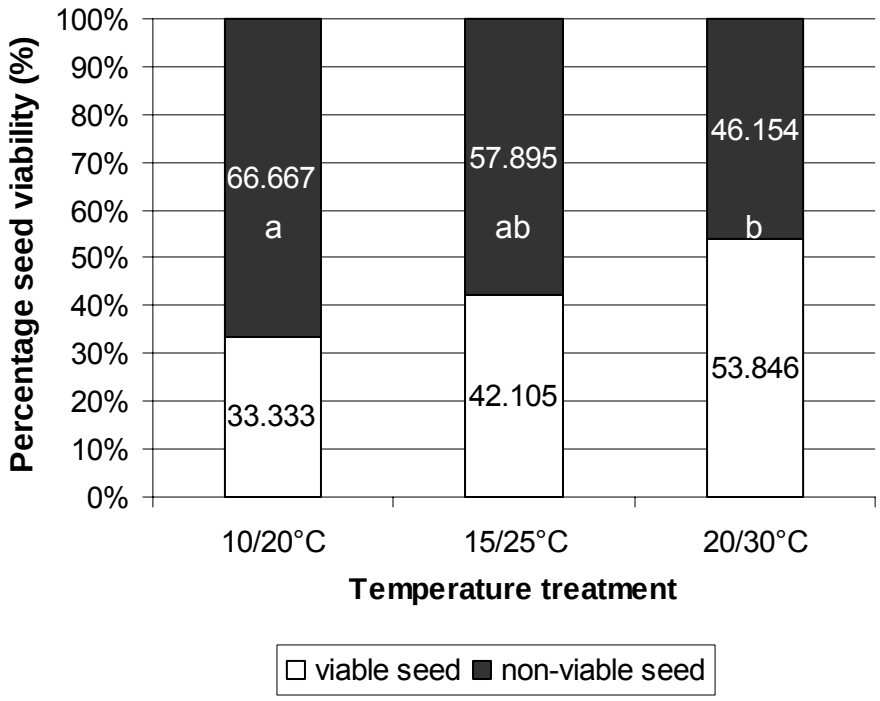

Figure 3.4 Percentage seed viability determined using the tetrazolium viability test of un-germinated *Moringa oleifera* seed from the rolled towel germination trial at the three temperature regimes. Treatment means with letters in common are not significantly different at $P \leq 0.05$.

Photos of various degrees of tetrazolium staining, and the criteria whereby seed viability was determined are illustrated in Figure 3.5.

Figure 3.5 The various degrees of tetrazolium staining, indicating the seed viability. A – unstained, non-viable seed. B – partially stained, nevertheless seed is considered non-viable. C – fully stained seed that is considered viable.

Figure 3.4 shows the differences in seed viability of ungerminated seed between the 10/20°C and 20/30°C regimes. However from the results in Table 3.1 the total seed viability did not vary between the three regimes. This is as a result of merely 12% of seed failing to germinate at the 10/20°C compared to the 26% at the high 20/30°C TR. Viability testing revealed that the greater proportion (66.7%) of ungerminated seed at the 10/20°C regime were non-viable, whereas less than half (46.2%) of the ungerminated seed at the 20/30°C regime were non-viable. The germination percentage at the 10/20°C regimes thus seemed higher, but in reality only 32.2% of the seeds remained ungerminated, compared the 53.8% at the 20/30°C TR. The reduced germination percentage at the 20/30°C TR was thus not as a result of inferior seed viability, but rather seed dormancy. This is an indication of induced dormancy at the higher TR or breaking of dormancy at the lower TR. It therefore appears as if *M. oleifera* seed and their consequent germination are affected by the dormancy-releasing effect of cold-stratification (Finch-Savage and Leubner-Metzger, 2006). Stratification is known to improve seed germination in various plant species

(Baskin, *et al*., 2001; Cavieres and Arroyo, 2000), for *M. oleifera* however, low temperatures merely increased the germination percentages, while germination uniformity and vigour decreased.

3.4.2 Seedling growth

Figure 3.6 *Moringa oleifera* seedling.

The germination of *M. oleifera* seed is hypogeal, meaning that the cotyledons remain beneath the soil surface, where they have been deposited (De Vogel, 1980). Successive to the germination trial, the seedlings emergence (Figure 3.6), growth and development under the three TRS were measured on a daily basis for a 40-day period. Figure 3.7, illustrates the average seedling height and growth rate at the

different TRS. From this data it is evident how seedling growth is enhanced by an increase in temperature. At the two lower TRS, the growth of the seedlings tend to demonstrate more or less linear growth, while under the high 20/30°C TR, growth is almost sigmodial. From the average seedling height after the 40-day period, seedlings germinated and grown at 20/30°C were on average 91.6% taller than those grown at 15/25°C, and 177% taller than the seedlings grown at the 10/20°C TR. The temperature effect on seedling growth both in the seedling trays as a whole and the individual seedlings is illustrated in Figure 3.8 and 3.9 respectively.

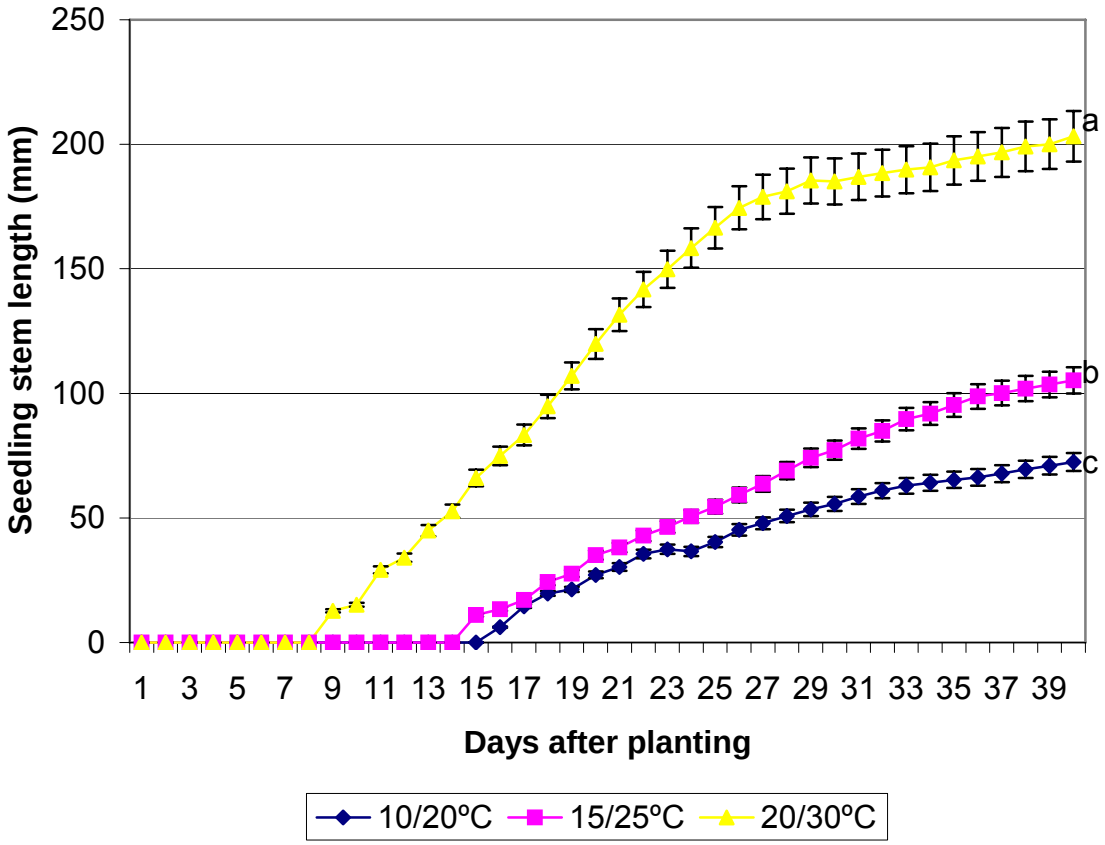

Figure 3.7 Differences in *Moringa oleifera* seedling stem growth under three temperature regimes over a 40-day period. Treatment means with letters in common are not significantly different at $P \leq 0.05$. Vertical bars represent LSD.

Differences in the average seedling height (Figure 3.7) between the three TRS were obtained at the end of the 40-day growth period.

Figure 3.8 Visual illustration of the growth differences of *Moringa oleifera* seedlings between the three temperature regimes after 40 days. The increase in growth is apparent from left to right with the increase in temperature regime.

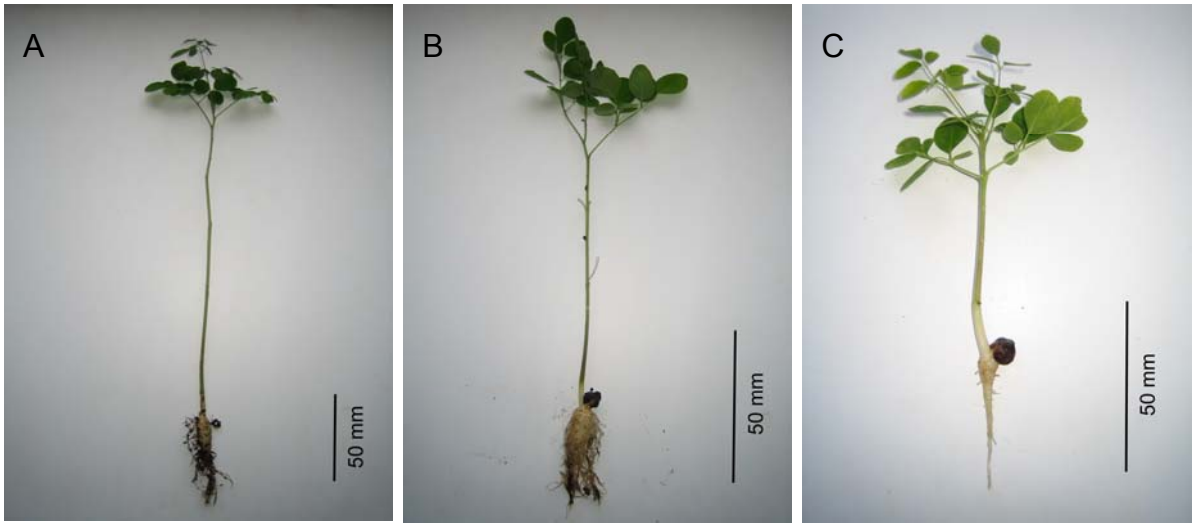

Figure 3.9 Photographic illustration of the differences in *Moringa oleifera* seedling size, after a 40 day exposure to the three temperature regimes. A – 20/30°C, B – 15/25°C and C – 10/20°C.

According to Roberts and Summerfield (1987), numerous plant species exhibit a positive linear relationship between temperature and seedling growth after emergence. The increase in growth of *M. oleifera* seedlings on the other hand is exponential with the increase in temperature (Figure 3.10). Plant growth increases rapidly between 10°C and 30°C, however beyond this temperature range, in either direction, growth decreases sharply. This is mainly due to changes in reaction rates of metabolic pathways (Downs and Hellermers, 1975).

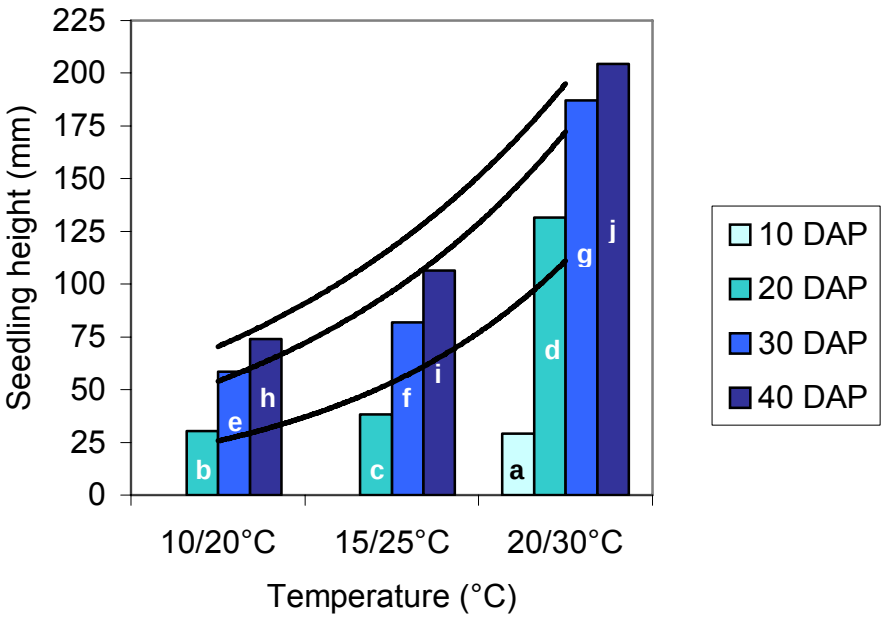

Figure 3.10 Exponential growth of *Moringa oleifera* seedlings with an increase in temperature regime. DAP – days after planting. Treatment means with letters in common are not significantly different at $P \leq 0.05$.

The effect of the three TRS on seedling leaf development, corresponds to that of seedling height (Figure 3.11). Similar to the effect of temperature on seedling height, the number of leaves that developed at the two lower TRS were relatively linear, while under the high 20/30°C TR, it was roughly sigmodial. Initial leaf development was significantly earlier (approximately seven days) under the 20/30°C TR, compared to both the lower TRS.

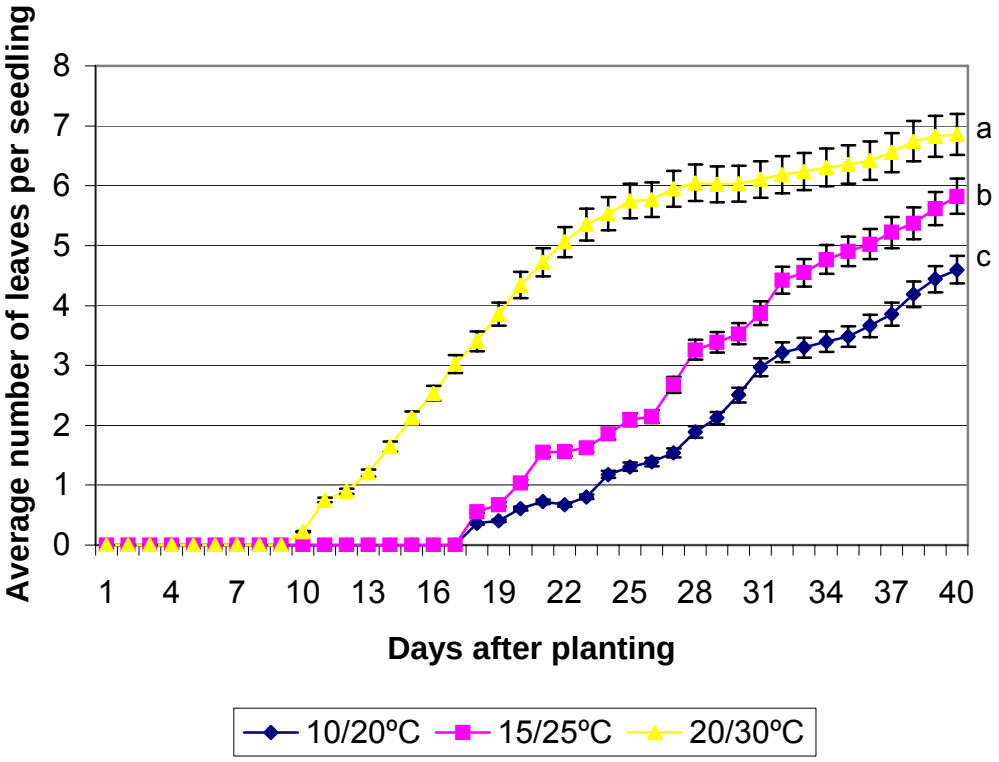

Figure 3.11 The average number of leaves per seedling over a 40-day period under the various temperature regimes of *Moringa oleifera*. Treatment means with letters in common are not significantly different at $P \leq 0.05$. Vertical bars indicate LSD.

From the results of the three TRS, the 20/30°C regime was found to be most favourable, as it facilitated the best seedling growth, with regards to growth rate and leaf development. The effects of even higher temperatures on seedling growth are still unclear, a facet that future studies should possibly look into.

The 20/30°C is thus a recommendable TR for both the germination and seedling growth of *M. oleifera.* According to Hartmann *et al.* (2002) it is vital to ensure that additional environmental conditions remain optimal, should germination and growth take place under high temperature conditions. Seedlings should thus receive sufficient moisture, light, fertilization and proper sanitation.

3.5 Conclusion

From these trials and the information gathered it is evident that although slightly higher germination percentages are achieved at the low 10/20°C TR, a lower MGT with a higher germination rate with better uniformity is achieved at a TR of 20/30°C. The 20/30°C TR also favours the consequent seedling growth, as the highest growth rate and leaf development were documented at this TR. Due to dormancy observed in seeds sown at the 20/30°C TR, an incubation period at a lower temperature of 10-20°C for 5-7 days should break seed dormancy, which would improve seed germination percentages. The subsequent recommended cultivation TR for seedlings prior to transplanting is 20/30°C, as this TR demonstrated the best results across all measured parameters.

3.6 References

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CHAPTER 4

TEMPERATURE EFFECT ON GROWTH AND DEVELOPMENT OF *MORINGA OLEIFERA* LAM. TREES.

4.1 Summary

Temperature is an important climatic factor influencing both geographical plant distribution and growth. While *Moringa oleifera* trees are naturally found in tropical climates around the world, 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 trees 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 favourable towards overall tree growth, as the highest values were obtained across all 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 3.09X (10/20°C), 1.44X (15/25°C) and 1.23X (20/30°C) compared to their non-hardened off counterparts.

The average tree leaf area increases followed a similar trend in both tree height and stem diameter, but expressed more volatility at the higher TRS. 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. This is in all probability the result of water stress, as the moisture demands of the rapidly increasing leaf area exceed the supply by the roots.

Temperature also influenced leaf thickness. Leaves from the 20/30°C treatment were on average 0.136 mm thick, compared to 0.239 mm at 10/20°C. This is a reduction of 43.1% as a result of a mere 10°C increase in temperature. Leaves were thicker mostly due to a broader spongy mesophyll layer.

4.2 Introduction

Moringa oleifera Lam. trees are found mainly in tropical and sub-tropical regions throughout the world due to their preference towards warmer growing environments (Jahn, 1988). Amongst all environmental factors affecting plant growth, temperature is one of the most important climatic factors. Not only does temperature influence tree physiology and development but also governs natural geographical plant distribution worldwide (Sakai and Larcher, 1987; Grace, 1988).

Considering the versatility of *M. oleifera*, the commercialization thereof would benefit from expansion of production areas into cooler climates. The performance of *M.*

oleifera trees at low growing temperatures is thus far unknown, and this prompted the investigation into tree growth across a range of TRS.

Overall tree growth is governed by two separate growth processes namely primary and secondary growth. Primary growth is responsible for the increase in tree height, while stem thickening is the result of secondary thickening growth (Lanner, 2002 ; Raven *et al.*, 1999). Tree height, stem diameter and leaf area expansion were therefore the measured parameters to quantify the effects of temperature on tree growth.

The main objective of this study was therefore to determine the effect of three TRS on growth and dry matter production of *M. oleifera*. The identification of specific temperature ranges suitable for *M. oleifera*, as well as the temperature limits beyond which satisfactory growth is unattainable, is essential for successful commercial production purposes and are currently still indefinite. Regions being ecologically suitable for consistent and satisfactory production would therefore be identifiable.

4.3 Materials and Methods

All growth trials described throughout this Chapter were conducted at Phytotron Section on the Experimental Farm of the University of Pretoria (25°45′ S, 28°16′ E) at an altitude of 1372m above sea level.

4.3.1 Tree height and stem diameter measurements

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 favourable greenhouse conditions between 20/25°C, 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. Equal numbers of hardened-off (132) and non hardened-off (132) 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, while the temperature inside the greenhouses had a permitted margin of ± 2°C from the set temperature, due to the influence of influx radiation. The abovementioned 264 trees were equally divided and randomly assigned to the three different TRS namely, 10/20°C, 15/25°C and 20/30°C, simulating night/day temperature fluctuations and exposed to natural daylight. Each tree was labeled and provided with a unique number to enable accurate monitoring of individual tree performance throughout the trial. The average PAR at 12:00 in the afternoon on a clear day, inside the temperature-controlled greenhouses was measured at 1350 $\mu mol.m^{-2}.s^{-1}$. Complete weather data for the entire trial period is given in the Appendix (Table 8.A3). To adjust nitrogen deficiencies evident from soil analysis results (Appendix, Table 8.A2), 20g of LAN (28) fertilizer was applied 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.

4.3.2 Leaf morphology and leaf area measurements

The total leaf area estimates of each tree, were calculated according to a non-destructive method of LAI calculation described by Siegfried *et al.* (2007). Firstly, the number of tripinnate leaves as well as the number of leaflets on the youngest mature leaf of every tree were 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, Licore 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.

Leaf conductance ($mmol.m^{-2}s^{-1}$) measurements at the three TRS were conducted 16 weeks after trial initiation using a Porometer (Model SC-1, manufactured by Decagon Devices Inc. 950 NE Nelson Ct. Pullman, WA 99163. USA).

The Photosynthetic Active Radiation (PAR) ($\mu mol.m^{-2}s^{-1}$) was measured using a ceptometer (AppPAR LP80 Series, manufactured by Decagon Devices Inc. 950 NE Nelson Ct. Pullman, WA 99163. USA).

The instant measurements of leaf chlorophyll content at the three TRS were conducted using a Minolta SPAD-502 chlorophyll meter (Minolta Camera Co., Ltd., Japan). Chlorophyll measurements were taken between the midrib and the leaf margin from leaflets randomly selected from the youngest mature leaf on trees within each greenhouse at 12:00 in the afternoon.

The stomatal index (SI), rather than stomatal density (SD) of the various treatments was determined according to the formula below developed by Salisbury (1927) for comparison, as the SD might be affected by the expansion of the surrounding epidermal cells due the factors such as light, temperature, leaf position and water status (Royer, 2000).

$$SI\ (\%) = \frac{\text{stomatal density}}{\text{stomatal density + epidermal cell}} \times 100$$

Light microscopy

Leaflet segments for anatomical examination were randomly collected from the three temperature treatments and prepared for light microscopy before being imbedded according to Coetzee and van der Merwe (1996). Segment specimens were fixed in 2.5% gluteraldehyde in a 0.075 M phosphate buffer (pH 7.4-7.6) for two hours, before being rinsed 3X in the same 0.075 M phosphate buffer (10 minutes each). Specimens were then fixed in 0.5% aqueous osmium tetroxide (OsO_4) for 2 hours and rinsed 3X with distilled water (10 minutes each), followed by dehydration of the

specimens in a range (30%, 50%, 70%, 90%) of ethanol:water dilutions, followed by three times in 100% ethanol. Leaf samples were then impregnated with 50% Quetol epoxy resin for 1 hour followed by 4 hours in 100% Quetol, and then polymerized at 60°C for 39 hours. Sections of 0.5 to 1 µm thick were cut with a Reichert Ultracut E ultramicrotome and placed onto microscope slides before being stained with toluidine blue. Sections were mounted in immersion oil and viewed with a Nikon Optiphod light microscope. Photographs were taken digitally using a Nikon DXM 1200 digital camera.

Scanning electron microscopy (SEM)

Randomly collected leaflets from the three different TRS were prepared for SEM according to Coetzee and van der Merwe (1996). A 3mm X 5mm square leaf sample was sectioned from the center of a pinnule (leaflet) (between the midrib and the pinnule margin) collected from a central pinna. The fixation and dehydration procedures of the leaf samples used for the SEM were identical to those discussed in the preceding light microscopy section. Following dehydration, some of the leaf samples were transferred and dried in a Bio-rad E3000 critical point drier with liquid $C0_2$, before being mounted on aluminum stubs and coated with gold in a Polaron E5200C sputter coater. Leaf sections were viewed with a JOEL 840 scanning electron microscope, while photographs were taken digitally.

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) to enable the comparison between treatment means.

4.4 Results

4.4.1 Tree height and stem diameter

The average tree height and stem diameter within each TR for the 32-week trial duration is illustrated in Figure 4.1. Amongst the three TRS investigated in this trial, the 20/30°C TR clearly was the most favourable for tree growth, with a final average tree height of 1,97m and stem diameter of 28.37mm. Growth at the 15/25°C TR was significantly less with a final average tree height and stem diameter of 1.10m and 16.11mm 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 0.48m and stem diameter to 8.75mm. 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.

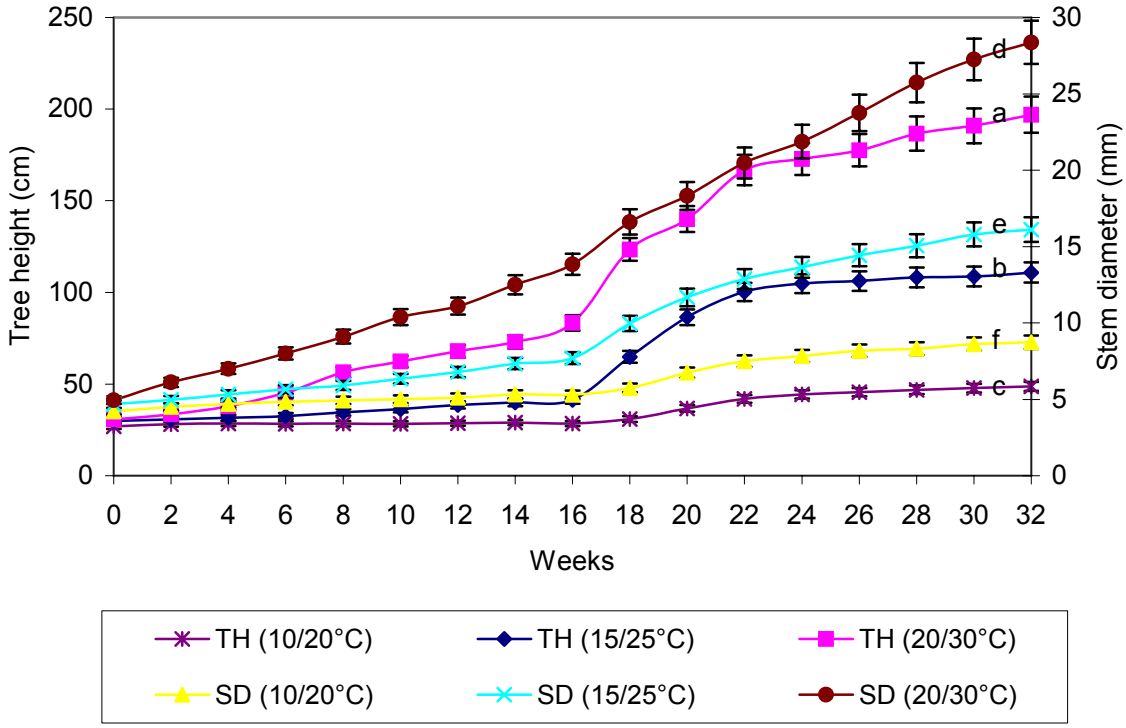

Figure 4.1 Differences in average tree height (cm) and stem diameter (mm) of *Moringa oleifera*, at three temperature regimes over a 32-week period. Treatment means with letters in common are not significantly different at $P \leq 0.05$. Vertical bars represent LSD (Least Significant Difference). TH – tree height and SD – stem diameter.

Significant statistical differences (P≤0.05) in average tree height and stem diameter (Figure 4.1) between the three TRS were already achieved as soon as two weeks after being placed at the various temperature treatments, and remained different for the remainder of the trial period.

Table 4.1 provides an overview of the average growth rates of the various treatments for the entire trial period. The growth rates between the various TRS were significantly different (P≤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.

Table 4.1. 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, TH – tree height and SD – stem diameter.

	Growth rate (mm/week)					
Pre-treatment	**10/20°C**		**15/25°C**		**20/30°C**	
	TH	SD	TH	SD	TH	SD
NHO	4.3 [a]	0.1 [g]	18.9 [c]	0.3 [i]	53.0 [e]	0.6 [k]
HO	13.4 [b]	0.2 [h]	27.1 [d]	0.3 [j]	65.1 [f]	0.7 [l]
Average	8.8 [ab]	0.2 [gh]	23.0 [cd]	0.3 [ij]	59.0 [m]	0.7 [n]

The differences in tree height at 7, 15 and 26 weeks after commencement of the trial under the three different TRS are illustrated in Figure 4.2 A, B and C respectively.

Figure 4.2 Difference in height of *Moringa oleifera* trees, due to the exposure to three different temperature regimes. A - 7 weeks B - 15 weeks and C - 26 weeks after trial initiation.

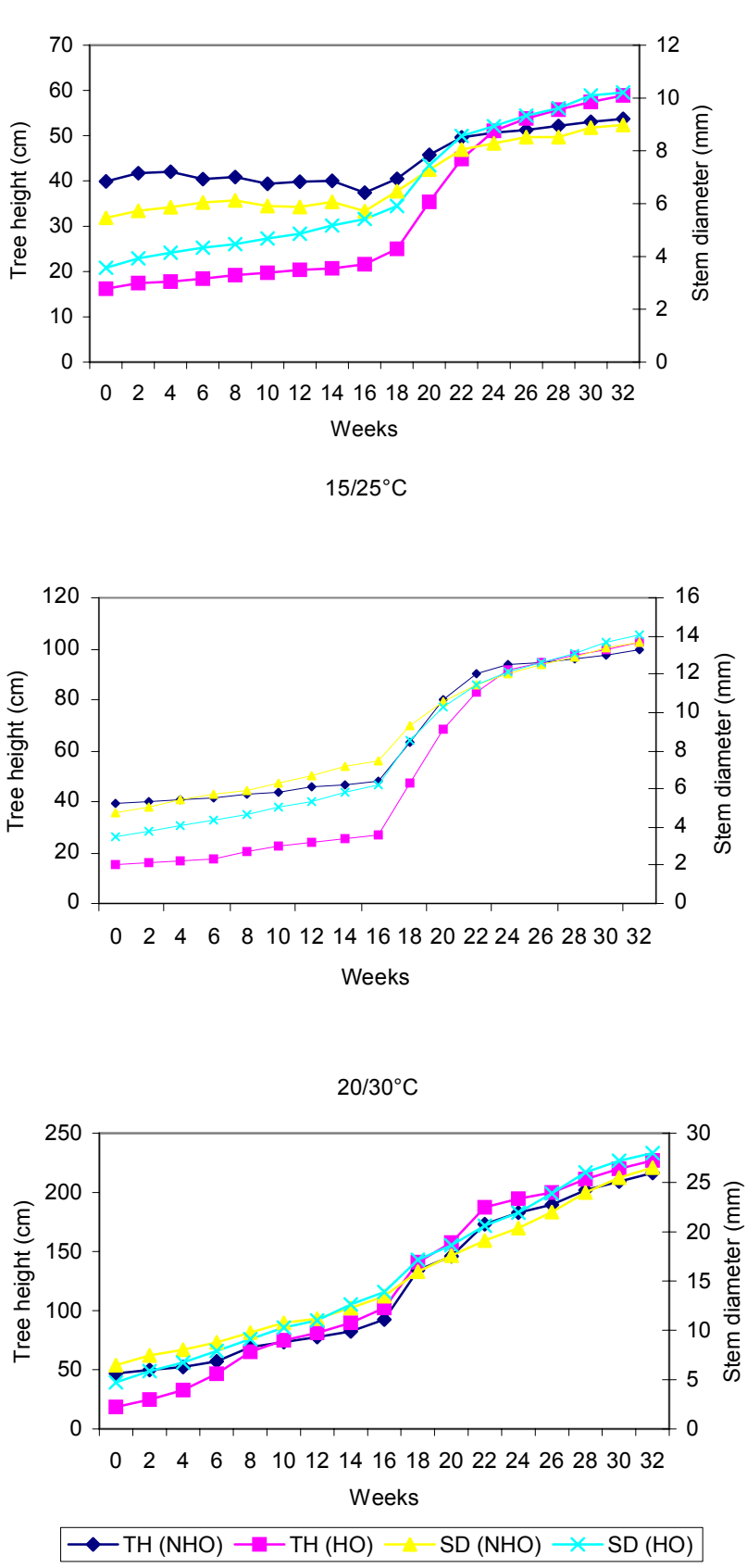

Figure 4.3 Differences in average tree height (cm) and stem diameter (mm) of *Moringa oleifera* cultivated from both hardened-off and non hardened-off seedlings at various temperature regimes over a 32-week period. NHO – non hardened-off, HO – hardened-off, TH – tree height and SD – stem diameter.

The differences between the hardened-off (HO) and non hardened-off (NHO) seedlings on the subsequent tree height and stem diameter increases at the three TRS over the 32-week trial period, is illustrated in Figure 4.3. Although growth varied greatly between the different temperature treatments, the HO seedlings demonstrated the highest growth rate (Table 4.1) as well as the highest final tree height and stem diameter, irrespective of the temperature treatment. However the percentage increase in growth rate between the three TRS, increased with the decrease in temperature. The hardening-off of seedlings thus becomes increasingly important with a reduction in temperature of the tree-growing environment. At the 10/20°C, 15/25°C and 20/30°C TRS, the respective growth rates of the non hardened-off seedlings were 67.6%, 30.5% and 18.7% lower for tree height and 47.1%, 15.3% and 13.8% lower for stem diameter, compared to their hardened-off counterparts.

Although the final tree height and stem diameter between the hardened-off and non hardened-off seedlings at all three TRS did not differ, the growth rates between the HO and NHO seedlings differed significantly among and within the three TRS.

Although the effect of the three TRS on tree growth is evident from the preceding sections, the correlation between the primary (tree height) and secondary growth (stem diameter) are illustrated in Figure 4.4. This demonstrates the direct relationship between primary and secondary growth as well as the similar response towards their growing environment.

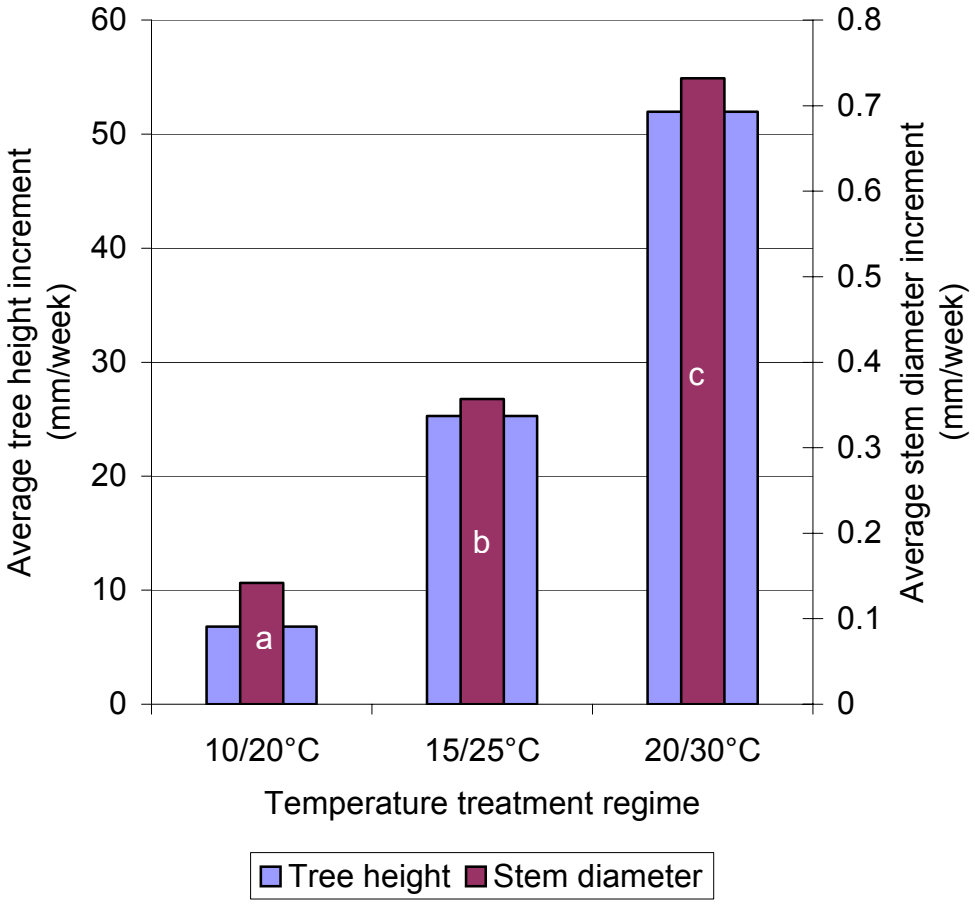

Figure 4.4 Response of both primary and secondary growth of *Moringa oleifera* trees towards the three temperature regimes. Different letters indicate significant differences at $P \leq 0.05$ according to the F-test.

4.4.2 Leaf morphology

4.4.2.1 Vegetative growth

Average leaf area (cm^2) estimates of the trees subjected to the three different TRS for the 32-week trial period are given in Figure 4.5. Although the average leaf areas of the trees at the three temperature treatments follow a similar trend to tree height and stem diameter discussed in the previous sections, the average leaf area estimates expressed more volatility. With the increase in temperature, greater leaf area fluctuations were observed, as trees would demonstrate cycles of more

frequent leaf drop followed by renewed flushes. The differences between high and low peaks were also accentuated by the size of the individual leaves. The 20/30°C treatment was most unpredictable (Figure 4.5), while the 15/25°C treatment did show volatility, but to a lesser extend than the 20/30°C treatment. The 10/20°C is clearly the most stable regime, showing a slight but constant increase in leaf area over the 32-week trial period.

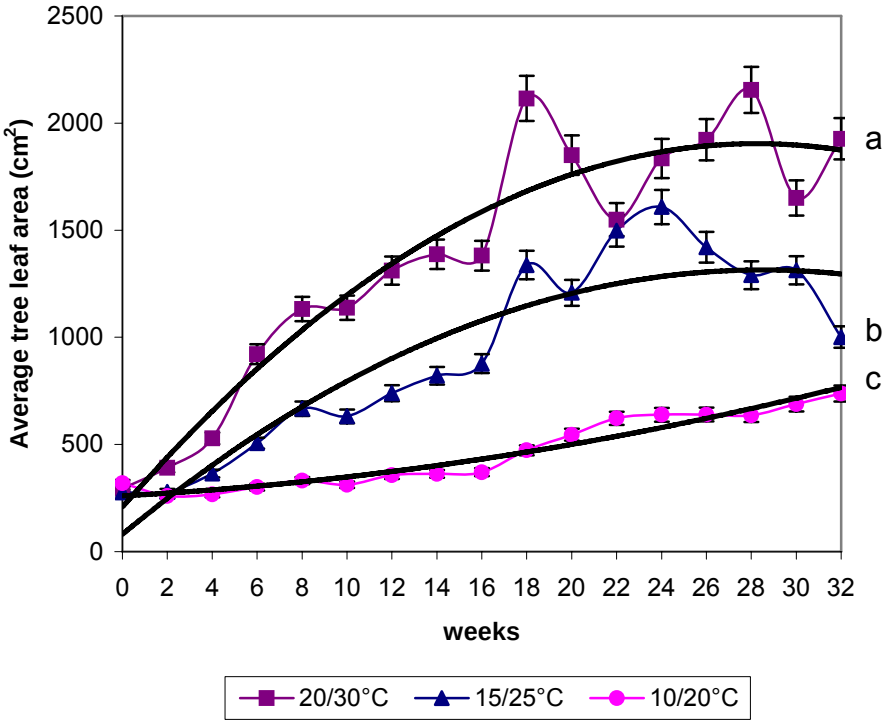

Figure 4.5 Increase in average tree leaf area (cm^2) of *Moringa oleifera* trees at three temperature regimes over a 32-week period. Treatment means with letters in common are not significantly different at $P \leq 0.05$. Vertical bars represent LSD.

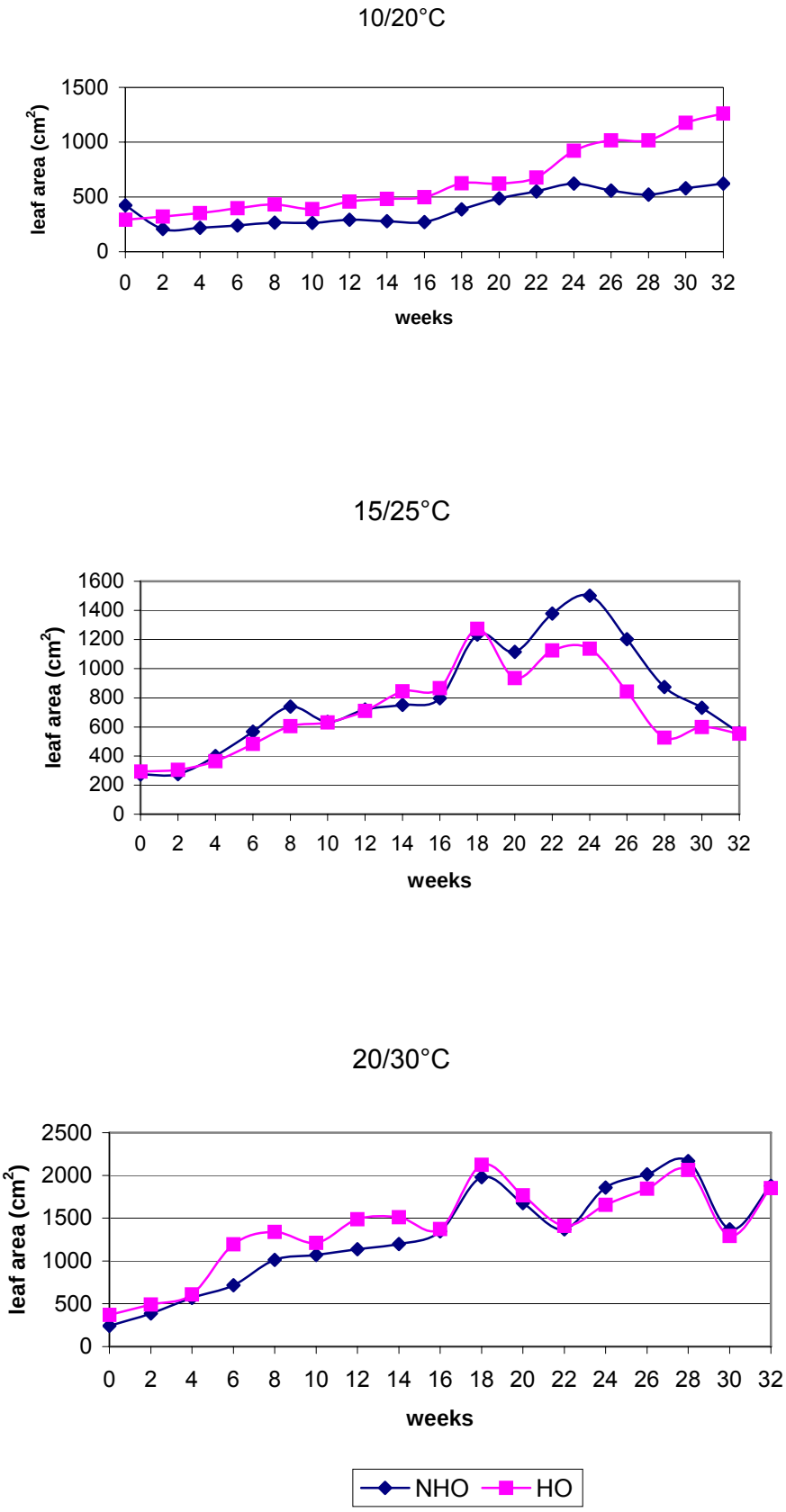

Figure 4.6 Comparison of average leaf area (cm^2) of *Moringa oleifera* trees cultivated from both hardened-off and non hardened-off seedlings at various temperature regimes over a 32-week period. NHO – non hardened-off and HO – hardened-off.

Figure 4.6 illustrates the effect that the hardening-off pre-treatment had on the consequent leaf area expansions through-out the subsequent trial period across all three TRS. The 10/20°C TR was the only regime showing a significant net increase in leaf area as a result of hardening-off. The final leaf area doubled (1260.72 cm^2) compared to the non hardened-off (620.14 cm^2) leaves. While the final leaf areas of both the 15/25°C and 20/30°C TRS were not affected by the hardening-off of the seedlings.

4.4.2.2 Leaf anatomy, chlorophyll content and conductance

The difference in TR also influenced leaf thickness, as leaf thickness decreased with an increase in temperature. Leaves randomly collected from the trees grown under the 20/30°C treatment, were on average 0.136 mm thick, while the average leaf thickness under the 10/20°C regime was 0.239 mm (Figure 4.7 & 4.8). The average leaf thickness was thus reduced by 43.1%, caused by a 10°C increase in the night/day TR. The leaves from trees grown at 20/30°C not only had fewer spongy mesophyll tissue, but also shorter palisade cells, compared to the leaves from the 10/20°C treatment. No significant differences ($P \leq 0.05$) in leaf thickness could however be established between HO and NHO trees within each TR.

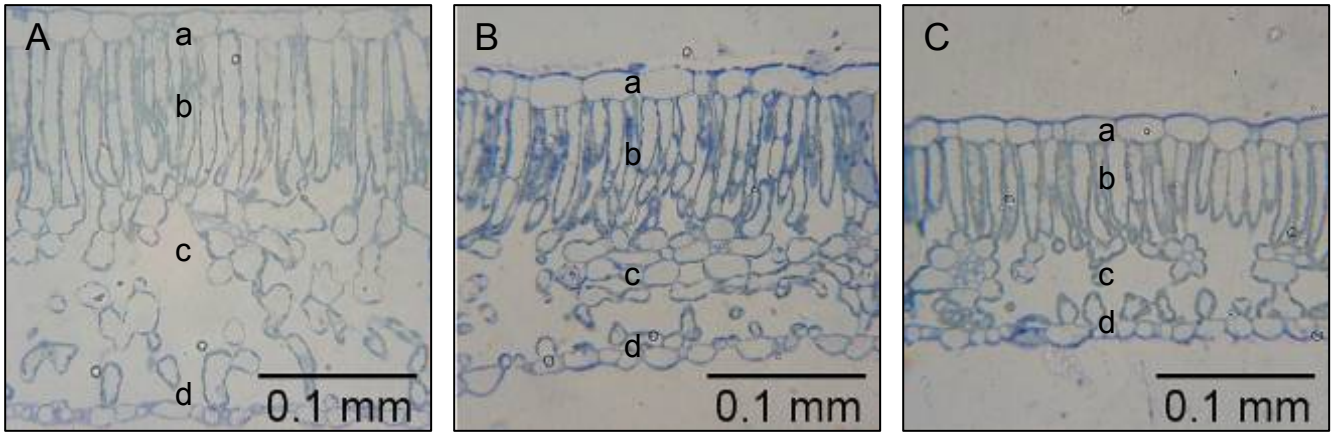

Figure 4.7 Differences in leaf anatomy caused by the different temperature treatments. A = 10/20°C, B = 15/25°C and C = 20/30°C. a – upper (adaxial) epidermis, b - palisade parchenchyma, c – spongy mesophyll, d – lower (abaxial) epidermis.

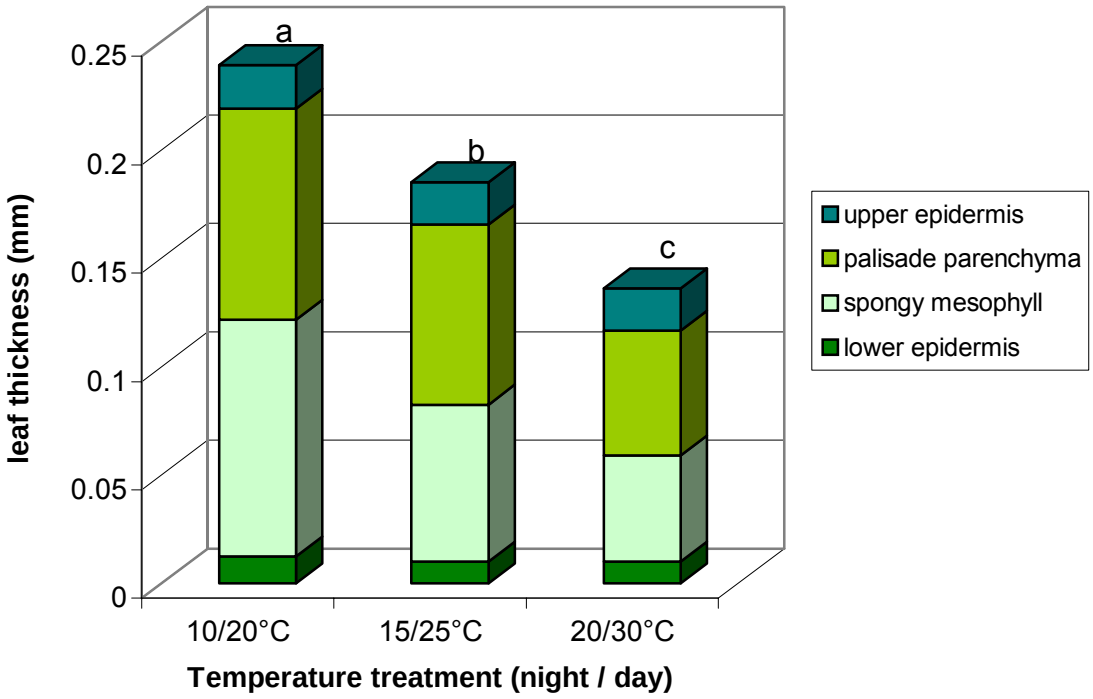

Figure 4.8 The temperature effect on *Moringa oleifera* leaf thickness and the various leaf components. Different letters indicate significant differences at $P \leq 0.05$ according to the F-test.

Further physiological differences were also revealed after a closer look at the abaxial leaf surfaces of trees from the three temperature treatments. SEM photographs of the abaxial leaf surfaces from the 10/20°C, 15/25°C and 20/30°C temperature treatments at 200X and 800X magnification are given in Figures 4.9. The number of stomata per unit leaf area was higher at the lower 10/20°C temperature treatment, however the individual stomata are smaller in size. Although the stomata are larger at the higher 20/30°C temperature treatment, the lower stomatal density (SD) results in a reduction in leaf conductance as illustrated in Figure 4.10.

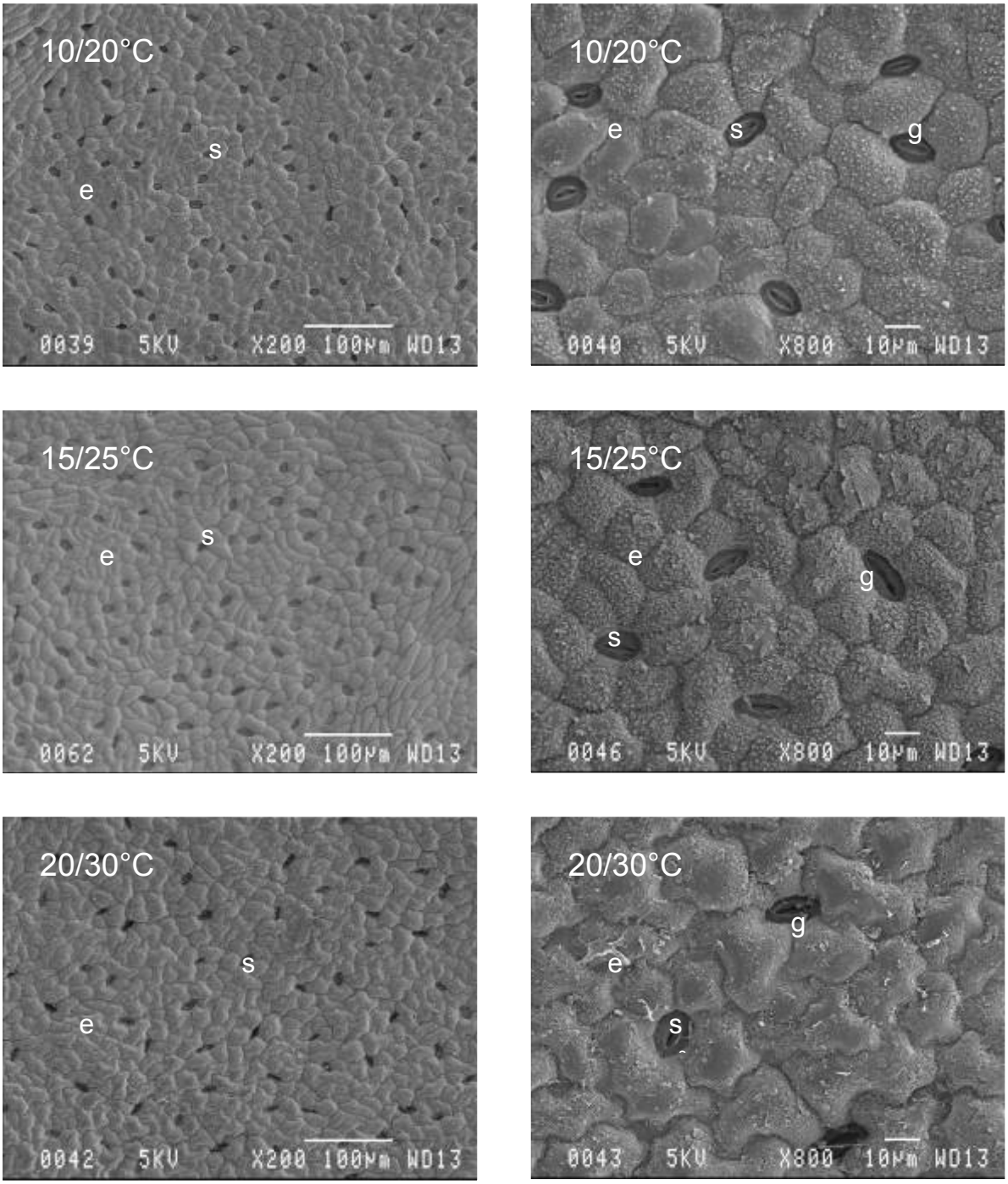

Figure 4.9 Illustration of differences in stomatal number and size between the 10/20°C, 15/25°C and 20/30°C temperature treatment at a 200X (left) and 800X (right) magnification. S – Stomata, E – Epidermal cells and G – Guard cells.

The average SI of the three temperature treatments was 11.56%, 10.21% and 8.49% for the 10/20°C, 15/25°C and 20/30°C treatments respectively. With a significant difference in SI between the high 20/30°C and low 10/20°C TR, no differences in the SI could however be found between the 10/20°C and 15/25°C as well as the 15/25°C and 20/30°C treatments. The lower 10/20°C treatment regime had more stomata and epidermal cells per unit area, compared to the higher 20/30°C temperature treatment regime. But although the guard cells of the stomata and epidermal cells were sizably larger at the higher temperature 20/30°C treatment, the SI was still lower, confirming the reduced leaf conductance measured at higher temperatures as illustrated in Figure 4.10.

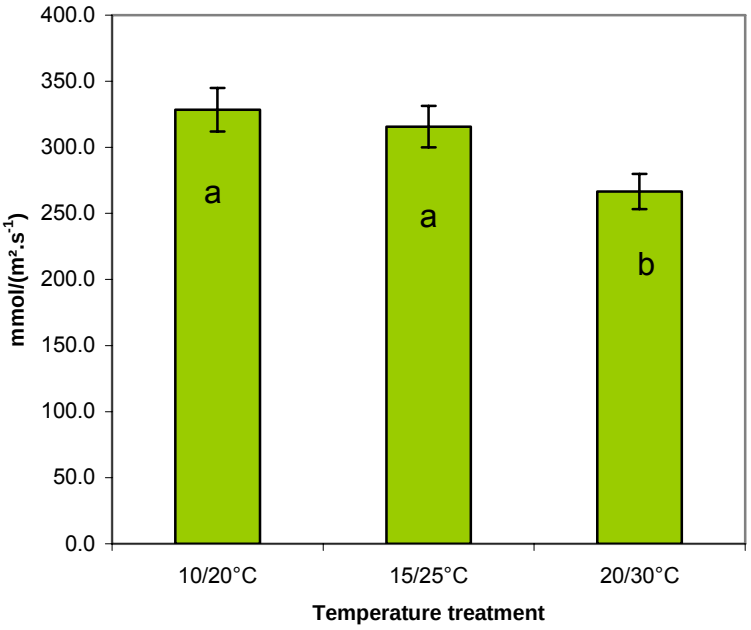

Figure 4.10 Differences in leaf conductance measured with a Porometer (mmol/m².s^{-1}) between the three temperature treatments, 16 weeks after trial initiation. Different letters indicate significant differences at $P \leq 0.05$ according to the F-test.

Given that both hydraulic conductively (mmol/m².s^{-1}) and SI were significantly lower at the 20/30°C compared to 10/20°C TR, whole-plant transpiration rates were calculated from total plant leaf area (m^2) and hydraulic conductively (mmol/m².s^{-1}) measurements. Seeing that leaf conductance measurements were once-off while the leaf area of each TR was estimated throughout the duration of the experiment, the transpiration rate for the entire trial period is merely an indication of the actual water loss. The average projected plant transpiration rate (mmol.s^{-1}) for all three TRS was calculated for the entire trial period and is illustrated in Figure 4.11.

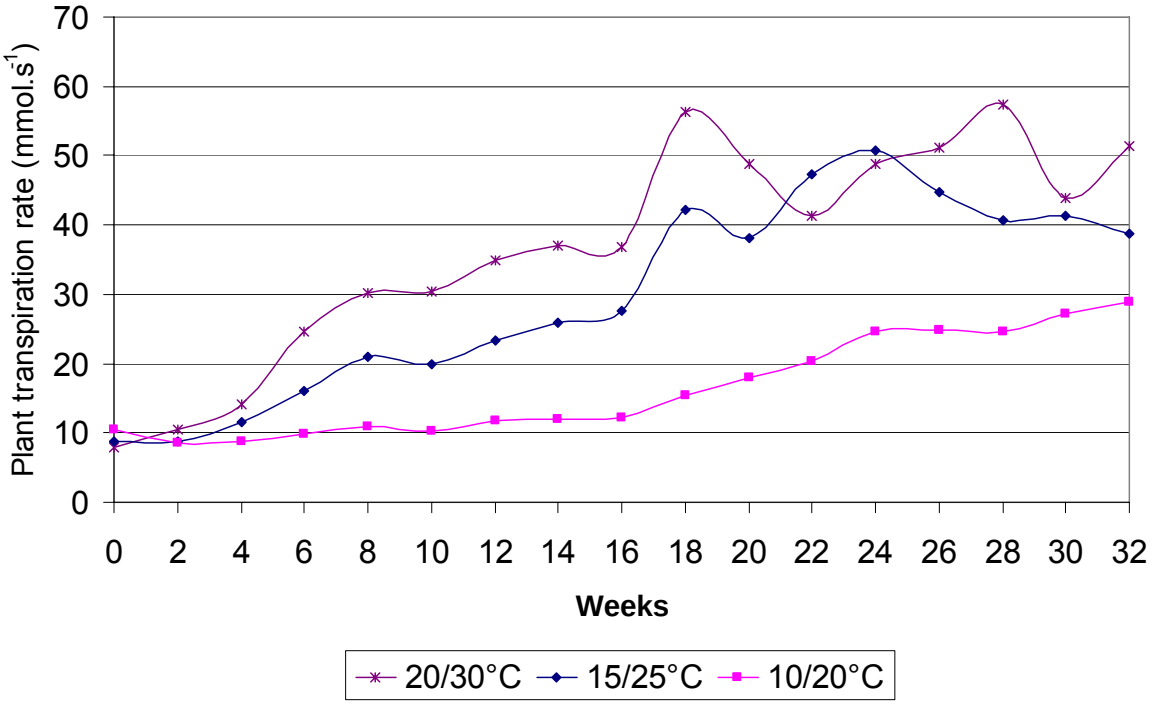

Figure 4.11 Relationship between plant transpiration rate (mmol.s^{-1}) and the three temperature regimes over the 32-week trial period.

Chlorophyll content measurements conducted at the three TRS revealed a decrease in leaf chlorophyll content with the increase in TR (Figure 4.12). According to Pinkard *et al*. (2006) and Loh *et al.* (2002) the chlorophyll content is linearly related to the leaf

nitrogen content. As the chlorophyll content is not only determined inexpensively but also much quicker using a portable chlorophyll meter, it can be used to estimate the leaf nitrogen instead of the conventional laboratory analysis (Wang, 2004). In an attempt to confirm the measured chlorophyll content using the SPAD-502 chlorophyll meter, the leaf nitrogen content was determined analytically. The similarity between both the chlorophyll and nitrogen content in response to the temperature treatments (Figure 4.12), confirms the reduction in chlorophyll content with the increase in TR. Though the chlorophyll content varied slightly between the TRS, neither the chlorophyll content nor the nitrogen content amongst the three TRS differed significantly (P≤0.05). Although there was a clear trend towards to a lower leaf chlorophyll content with the increase in TR.

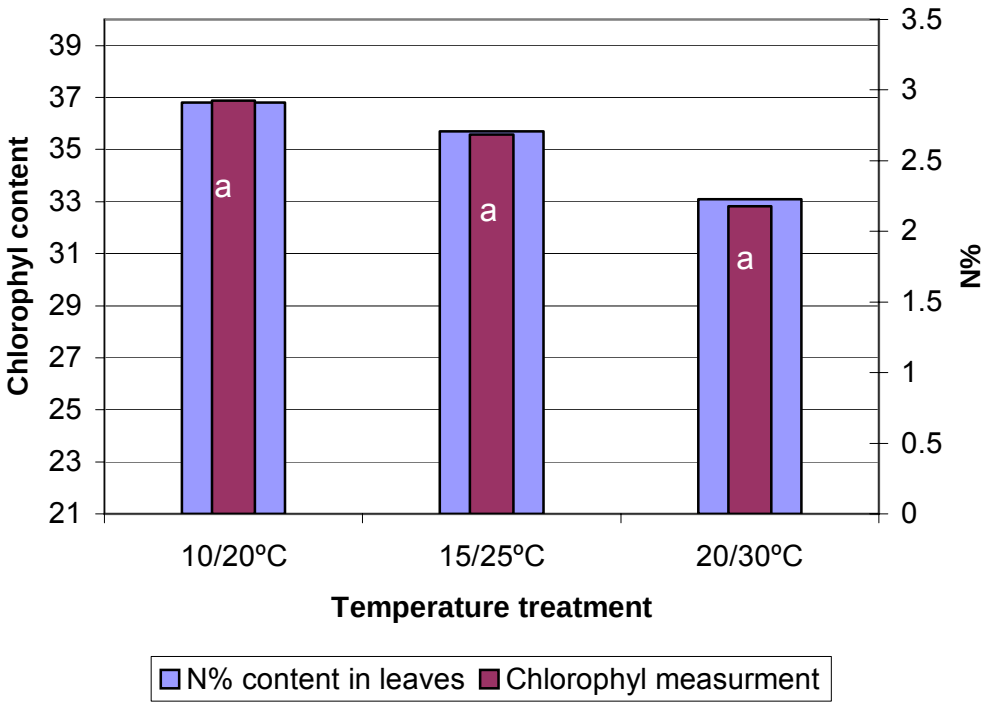

Figure 4.12 Differences in leaf chlorophyll content of *Moringa oleifera* trees measured with a SPAD-502 chlorophyll meter and leaf nitrogen content (%) form plant analysis results, between the three temperature treatments. Measurements were taken 16 weeks after trail initiation. Different letters indicate significant differences at $P \leq 0.05$ according to the F-test.

4.5 Discussion

4.5.1 Tree height and Stem diameter

The increase in TR brought about the increase the vegetative growth of *M. oleifera* trees. This agrees with observations made in several other tropical and subtropical trees (Menzel and Paxton, 1985; Trochoulias and Lahav, 1983; George and Nissen, 1987; Utsunomiya, 1992), such as mango (Whiley *et al.*, 1989), macadamia (Lahav and Trochoulias, 1982) and lychee (Menzel and Paxton, 1985).

Downs and Hellermers (1975) found that lower ambient growing temperatures decrease the partial pressure inside plant leaves, due to reduced molecular kinetic energy. This in turn lowers the vapor pressure of water inside the leaf, reducing transpiration, which in turn influences the movement of both organic and inorganic molecules throughout the plant. Turner and Lahav (1985) found that the nutrient absorption in bananas increased with the increase in temperature, with the degree of uptake being different for each of the various nutrient elements. They furthermore found that the nutrient use efficiency decreased in plants with an increase in temperature. The temperature effect on nutrient uptake would explain the difference in response to the fertilizer application observed in tree height and stem growth.

It is thus obvious that the growth and development of *M. oleifera* trees is immensely affected by the variation in temperature. According to Downs and Hellermers (1975), 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 coincide with the requirements of these enzymatic reactions. Since the enzymatic reactions responsible for the processing of photosynthates is temperature sensitive, growth and development are a function of the growing temperature. Therefore, temperature extremes would 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), 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. Lower root mass as a result of reduced dry matter production was measured in trees from the 20/30°C regime (Chapter 5), while reduced flowering was also observed and is discussed in Chapter 6. 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 10-20°C range. This not only verifies the fact that *M. oleifera* favours tropical growing environments as stated by Morton (1991) and Mughal *et al*. (1999), but also demonstrates the reluctance of *M. oleifera* to acclimatize and generate satisfactory growth under cooler climates.

The hardening-off process involves several complex processes that have an effect on numerous morphological and physiological mechanisms enhancing plant growth under unfavourable environmental conditions (Villar-Salvador *et al.*, 1999). 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) and Bañon *et al.* (2006) respectively. Both these papers reported the superiority of the hardened plants over their non hardened counterparts upon the exposure to unfavourable environmental growing conditions (Sánchez-Blanco *et al.* 2004; Bañon *et al.* 2006). The final growth between the HO and NHO seedlings at all three TRS for both the tree height as well as the stem diameter did not differ from one another. However, the growth rates between the HO and NHO seedlings for both these parameters differed significantly among and within the three TRS. 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 climates. Regardless of the final growing climate, the HO seedlings are at an advantage and is the hardening-off process thus highly recommended for *M. oleifera* trees.

4.5.2 Leaf morphology

4.5.2.1 Vegetative growth

Similarly to the temperature effect on both tree height and stem diameter, the average leaf area (cm^2) increased with an increase in TR. 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 extend 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.

The reduction in leaf area was caused by the senescence of the physiologically older leaves, presumably as a result of water stress (Fischer and Kohn, 1966). As both the irrigation frequencies and temperatures remained unchanged within all the temperature-controlled greenhouses, they were not solely responsible for the leaf abscission. Under both the 20/30°C and 15/25°C TRS the initial leaf abscission followed after the rapid leaf area increases subsequent to the nitrogen fertilizer application. The rapid leaf area increases, increased the stomatal number per unit leaf area, consequently increasing transpiration. Transpiration in turn, is directly linked to plant water consumption (Stanhill and Albers, 1974). The acute increase in tree leaf area, substantially increased their water requirements, as illustrated by the plant transpiration rates ($mmol.s^{-1}$) in Figure 4.11. Contrary to the lower stomatal count and hydraulic conductivity measured in leaves at the 20/30°C TR, the transpiration rate ($mmol.s^{-1}$) increased with rise in TR. Leaf area is therefore the single factor responsible for the increased water loss through transpiration with the increase in temperature, as both stomatal count and leaf conductance were considerably lower per unit area. The increased tree water consumption presumably exceeded the supply of the otherwise sufficient soil moisture levels under conditions of lower leaf areas. In an attempt the minimize water losses plants initially instigate stomatal closure as a short-term resolution, but if the water stress however persists, plants resort to leaf shedding to lower transpirational water loss (Peake *et al.*, 1975). Once stability between the leaf area and the soil moisture level that is able to sustain

the water demand at that leaf area is attained, the leaf area yet again gradually increases.

4.5.2.2 Leaf anatomy, chlorophyll content and conductance

Leaf thickness as well as chlorophyll content progressively decreased with an increase in TR. The same tendency was also observed in cherimoya (*Annona cherimola* Mill.) (Higuchi, 1999), macadamia (*Macadamia integrifolia*) (Trochoulias and Lahav, 1983) avocado (*Persea americana* Mill.) (Lahav and Trochoulias, 1982) and mangosteen (*Garcinia mangostana* L.) (Wiebel *et al.*,1994) trees. According to Higuchi (1999) this reduction in leaf thickness and the accompanied reduction in chlorophyll content is not the effect of varying light intensities, but rather the result of elevated air temperatures that plants are exposed to. Higher temperatures result in reduced development of mesophyll tissue, and thereby reducing the leaf thickness. This is a response of plants to minimize the damaging effects of photoinhibition. Photoinhibition occurs when the rate of light energy absorption exceeds the rate of its consumption in chloroplasts, resulting in damage to PSII caused by the excess of absorbed light energy (Melis, 1999). Exposure of plants to low temperatures can instigate photoinhibition, even under low to normal light intensities (Öquist and Huner, 1990 ; Schöner and Krause, 1990 ; Somersalo and Krause, 1989). Boese and Huner (1990) presume that the increase in mesophyll tissue responsible for leaf thickening at lower temperatures, may counteract photoinhibition. As the added palisade cells absorb an additional fraction of the light, thereby reducing the proportion of cells exposed to the high light intensity. The significantly thicker leaves of the 10/20°C TR are thus an adaptation to counteract photoinhibition.

The reduction in stomatal conductance with an increase in temperature, is partially the result of the lower SI at the higher TRS. However (Menzel and Simpson, 1986) found the increase in air temperature to raise the leaf to air vapor pressure deficit, thus lowering stomatal conductance in lychees. The decrease in stomatal conductance is thus due to both the difference in leaf physiology as well as environmental circumstances. The reduction in leaf conductance lowers photosynthesis, as photosynthesis is positively related to stomatal conductance (Kelly and Latzko, 1993). Considering the reduced stomatal conductance under the high 20/30°C TR, expectably trees at this regime would demonstrate reduced growth due to sub-optimal photosynthesis. However the far greater leaf area of trees at this TR increases the total number of stomata per plant, resulting in increased vegetative growth (Bañon *et al.*, 2006). Thus although the individual stomata might have had a lower conductance, the greater number of stomata per plant give the 20/30°C TR the advantage over the lower TRS.

The lower chlorophyll content at higher temperatures might be disadvantageous to photosynthetic performance, due to the reduced light use efficiency of the leaves (Higuchi *et al.*, 1999). However the larger leaf area at the higher TRS, overrided the disadvantages of reduced photosynthesis, resulting in a higher overall CO_2 assimilation rate.

4.6 Conclusion

Growth of *M. oleifera* is evidently favoured by high growing temperature of >25°C, confirming the preference 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 at low temperature environments.

4.7 References

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CHAPTER 5

EFFECT OF PACLOBUTRAZOL ON *MORINGA OLEIFERA* LAM. GROWTH AT THREE TEMPERATURE REGIMES.

5.1 Summary

The effect of paclobutrazol (PBZ) on growth of *Moringa oleifera* Lam. seedling trees is indistinct, and is exceedingly influenced by the growing environment of the tree. Although PBZ is predominantly used as a growth retardant for numerous other tree species, the only growth reduction in *M. oleifera* was observed at the low 10/20°C temperature regime (TR). At both the higher temperature regimes (TRS) namely 15/25°C and 20/30°C, growth was significantly higher, due to possible high temperature and water stress alleviation, induced by PBZ. Despite the futility of using PBZ as a growth retardant on *M. oleifera* in warm to hot growing climates, its effectiveness as a plant protector against several adverse environmental stresses seems justified. In addition to the exceptional drought tolerance of *M. oleifera*, PBZ could provide additional protection against the damaging effects of high growing temperatures.

5.2 Introduction

Various Plant Growth Regulators (PGR) are used in tree crops to promote flowering by shortening their juvenile phase, one such PGR is paclobutrazol (PBZ), commonly used for its flower induction properties in woody angiosperms (Meilan, 1997). PBZ

was selected as PGR, given that according to Nørremark and Andersen (1990), PBZ can either delay or promote flowering, depending on plant species. PBZ, a triazole, influences nearly all plants, by reducing stem elongation due to its extreme chemical activity (Barrett, 2001).

Gibberellins are plant growth hormones responsible for shoot elongation and are produced by the plant through the process of gibberellic acid (GA) biosynthesis. The application of PBZ inhibits GA biosynthesis as it blocks the oxidation of *ent*-kaurene, and subsequently stunts plant growth (Sponsel, 1995; Hartmann, 2002; Dole and Wilkins, 1999). According to Abdullah *et al.* (1998) caution should be taken as excessive stunting and failure to flower might be the result of application concentrations being too high. PBZ applied as a foliar spray is absorbed through both the leaves and stems before being transported via the xylem towards the growing tip (White, 2003).

The objective of this study was to determine the effect of both PBZ and temperature on the growth and development of *M. oleifera*. This Chapter primarily aims to provide an indication on the response of *M. oleifera* to the exposure of PBZ at a single recommended concentration, while also considering temperature as an additional factor influencing PBZ efficacy.

5.3 Materials and Methods

The preparatory steps described in Chapter 4 also apply to this Chapter. Once trees have been placed into the three temperature controlled greenhouses, 50% of the

trees within each of the three TRS were treated with PBZ. The PBZ (Paclobutrazole 250) used in this trial was sourced from, R.T Chemicals c.c., Pietermaritzburg, KZN, South Africa) [(±)-(R*,R*)-β-((4-chlorophenyl)methyl)-α-(1,1,-dimethylethyl)-1H-1,2,4,-triazole-1-thanol)] (Figure 5.1). According to Dole and Wilkins (1999), reduced efficacy was observed whilst using PBZ as a soil drench in a bark medium, as the PBZ became absorbed and reduced plant uptake. This prompted the foliar application of the PBZ rather than a soil drench since trees used in this trial were cultivated in a bark medium. From trial commencement, a foliar spray application of PBZ, using a hand-held pressurized sprayer, was repeated every 6 weeks. Due to the lack of specific application rates for *M. oleifera*, the application rate and volume recommended for "Landscape woody shrubs" by Basra (2000) were used. PBZ at a concentration of 100 mg.liter^{-1} (active ingredient) was evenly applied as a foliar spray at an application rate of 250 ml/m^2, with the tree density being 16 trees/m^2. The effect that the PBZ had on growth across the three TRS was determined from the bi-weekly measurements (tree height, stem diameter and leaf area) collected throughout the 32-week trial period.

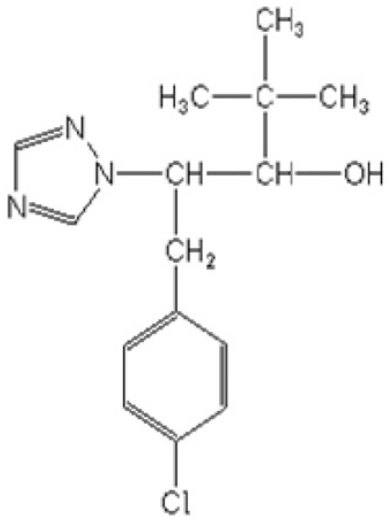

Figure 5.1 The chemical structure of paclobutrazol (Milfont *et al.*, 2008).

At trial termination 48 trees (8 trees from each of the 6 temperature and PBZ/control treatments) were randomly selected and partitioned into roots, stems and canopy. After determining the fresh mass of the various tree components, they were placed into an oven at 65°C. The leaves and stems were dried for 72 hours, while the tuberous roots were left to dry for one week, after which their dry mass was determined.

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) to enable the comparison between treatment means.

5.4 Results and Discussion

5.4.1 Effect of paclobutrazol on stem growth

Tree height (Figure 5.2) as well as stem diameter (Figure 5.3) growth were affected by both, temperature as well as the PBZ. This interaction between temperature and PBZ is indicative of the influence temperature has on the efficacy of PBZ. The effect of the fertilizer application during week 16 was responsible for the change in growth line gradient.

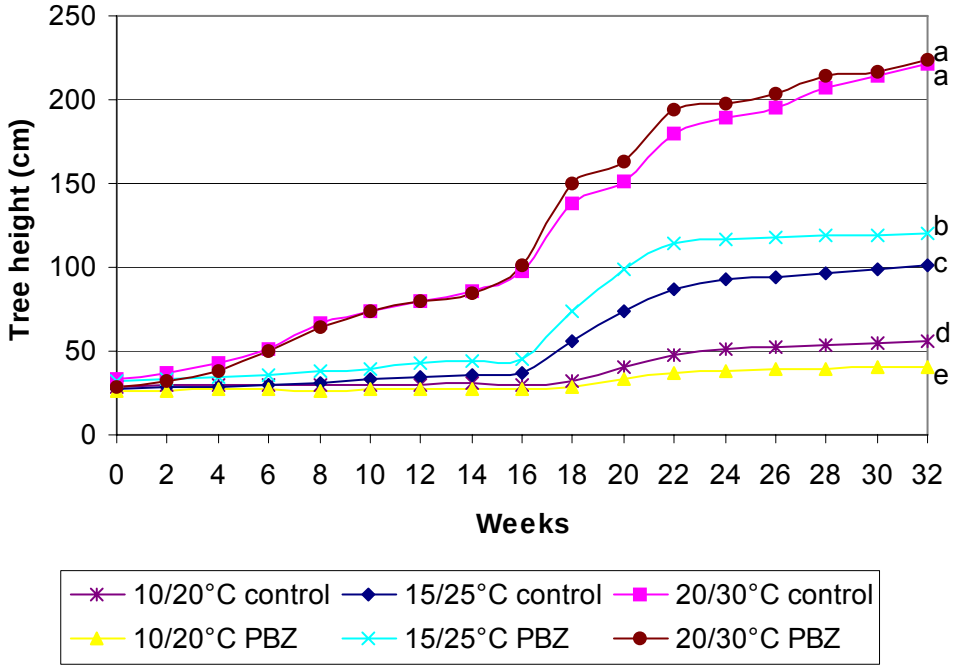

Figure 5.2 Paclobutrazol (PBZ) effect on average tree height (cm) of *Moringa oleifera* at the three temperature regimes over a 32-week period. Treatment means with letters in common are not significantly different at $P \leq 0.05$. The sudden increase in growth rate at week 16, was due to a nitrogen fertilizer application.

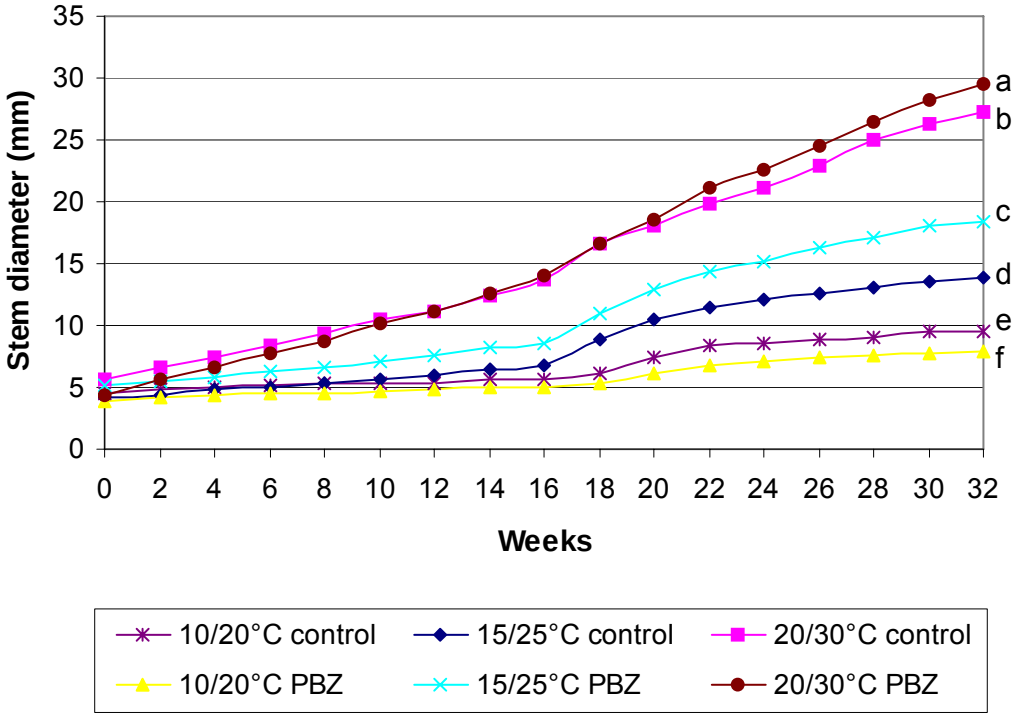

Figure 5.3 Paclobutrazol (PBZ) effect on average stem diameter (mm) of *Moringa oleifera* at the three temperature regimes over a 32-week period. Treatment means with letters in common are not significantly different at $P \leq 0.05$. The sudden increase in growth rate at week 16, was due to a nitrogen fertilizer application.

Tree height was influenced by the PBZ application within all three TRS, either positively or negatively. Trees treated with PBZ under the 10/20°C TR demonstrated a 46.2% reduction in growth rate compared to the control treatment. The growth rate of PBZ treated trees at the 15/25°C TR was on average 19.9% higher than those of the control treatment, while PBZ induced a non-significant tree growth rate increase of only 3.5% under the 20/30°C TR. Differences in final tree height between the PBZ and control treatment were found to be significantly different at both the 10/20°C and 15/25°C TRS (Table 5.1). Despite considerable growth increases measured in the PBZ treated trees from week 20 to 28 at the 20/30°C TRS, no difference as measured in the final tree height. Increases in tree height of PBZ treated trees at the 15/25°C regimes were continuous whereas PBZ only temporarily, but significantly increased growth in trees of the 20/30°C TRS. The typical growth retardation expected from a PBZ application was only evident at the low 10/20°C TR, while tree height of both the 15/25°C and 20/30°C regimes was unexpectedly higher. Table 5.2 illustrates the effect of the PBZ application and the significance thereof on the average growth rate (mm/week) for the three TRS. From this data the only noteworthy difference in growth rate (mm/week) between the PBZ and control treatment was observed at the low 10/20°C TR. Although the final growth at the 15/25°C TR was higher, the increase in average growth rate (mm/week) of the PBZ treatment for the entire trial period was found non-significant at $P \leq 0.05$.

Results from the PBZ treatment on stem diameter are illustrated in Figure 5.3. Twenty-six weeks since trial initiation the increase in average stem diameter of the PBZ treated trees for the 15/25°C and 20/30°C TRS became significantly higher than those of the untreated trees while PBZ reduced the final stem diameter at the

10/20°C TR (Table 5.1). Compared to the untreated trees, a 21.3% reduction in net stem diameter growth was measured at the 10/20°C TR as a result of the PBZ application whilst net stem growth increases of 35.0% and 34.7% were measured under the 15/25°C and 20/30°C respectively. No differences in average stem diameter growth rate (mm/week) for the 32-week trial period at the 10/20°C TR were found (Table 5.2). It is therefore apparent that the effect of PBZ on both tree height and stem diameter growth is temperature dependant. While stem length/diameter under the 10/20°C TR were reduced by PBZ, tree stem diameter at both the higher TRS (15/25°C and 20/30°C) was considerably higher. However tree height was significantly higher at only the 15/25°C TR.

Given that tree height growth is a direct function of the internode length, the effect of both temperature and PBZ on the average internode length (Figure 5.4) and tree height (Figure 5.2) are comparable. The differences in average internode length between the three TRS are illustrated in Figure 5.4. Synonymous to the differences in tree height at trial termination, PBZ reduced the internode length at the 10/20°C regime, while significantly increasing the length of the internodes at the 15/25°C TR. No statistical differences at $P \leq 0.05$ could be established between the treatments at the 20/30°C TR.

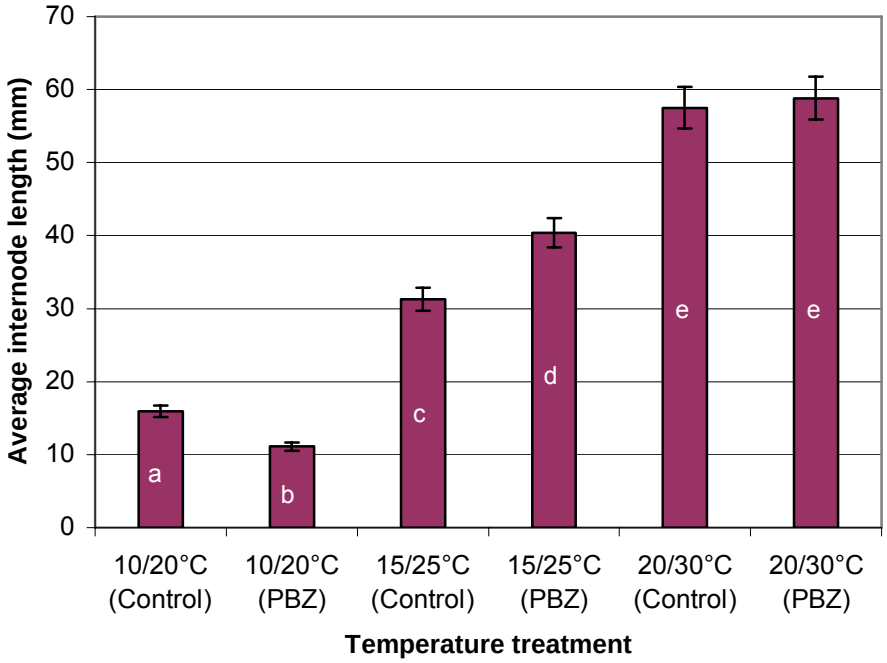

Figure 5.4 Variation in average internode length (mm) of *Moringa oleifera* between the paclobutrazol (PBZ) and control treatment at the three temperature regimes over a 32-week period. Treatment means with letters in common are not significantly different at $P \leq 0.05$.

5.4.2 Effect of paclobutrazol on leaves

The variation in average leaf area (cm^2) of *M. oleifera* trees at the three TRS are given in Figure 5.5. The leaf area response to the PBZ is comparable to that of both tree height and stem diameter, reducing leaf area at the low 10/20°C TR while increasing leaf area at both the two higher regimes. However, contrary to tree height and stem diameter, the leaf area increases were not as linear and consequently less predictable, especially at the two higher TRS. (An explanation and discussion on this phenomenon can be found under section 4.5.2.1 in Chapter 4). Despite the differences in leaf area between TRS, no differences were observed at the 10/20°C and 20/30°C TRS between the treated and non-treated trees (Table 5.1 & 5.2). When considering the average leaf area increases (cm^2/week) the only significant

increases in leaf area subsequent to the PBZ application was observed at the 15/25°C TR.

Despite non-significant differences in the number of leaves formed between the control and PBZ treated trees within each TR, the number of leaves amongst the three TRS differed noticeably from one another as illustrated in Figure 5.6.

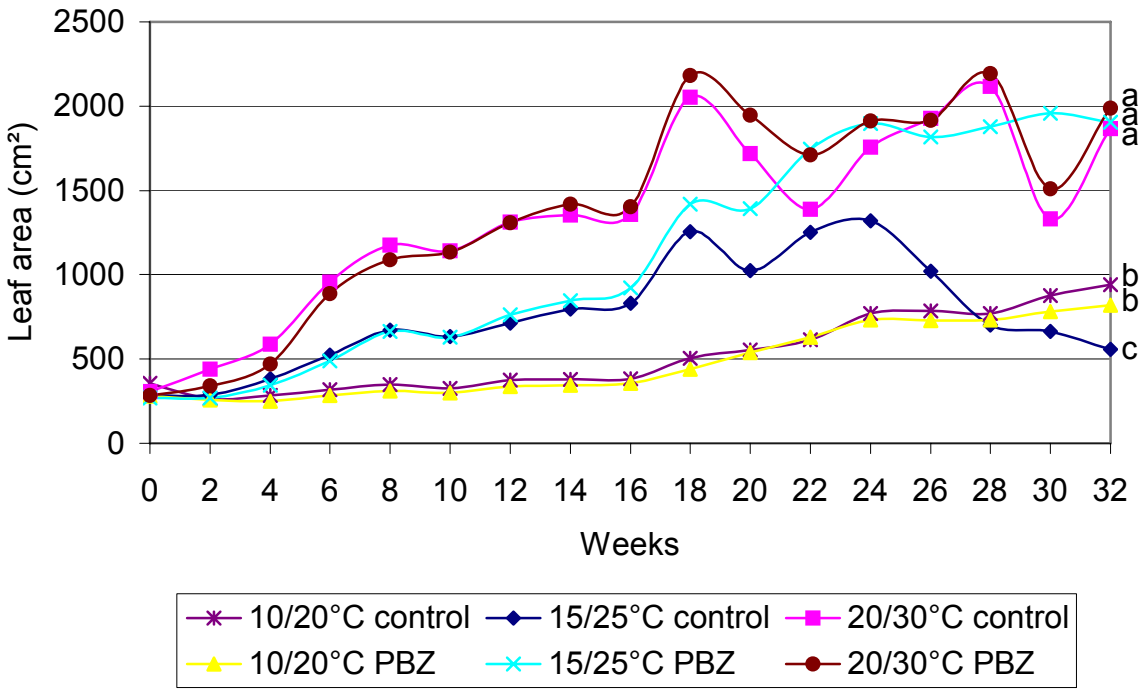

Figure 5.5 Paclobutrazol (PBZ) effect on average leaf area (cm^2) of *Moringa oleifera* at the three temperature regimes over a 32-week period. Treatment means with letters in common are not significantly different at $P \leq 0.05$.

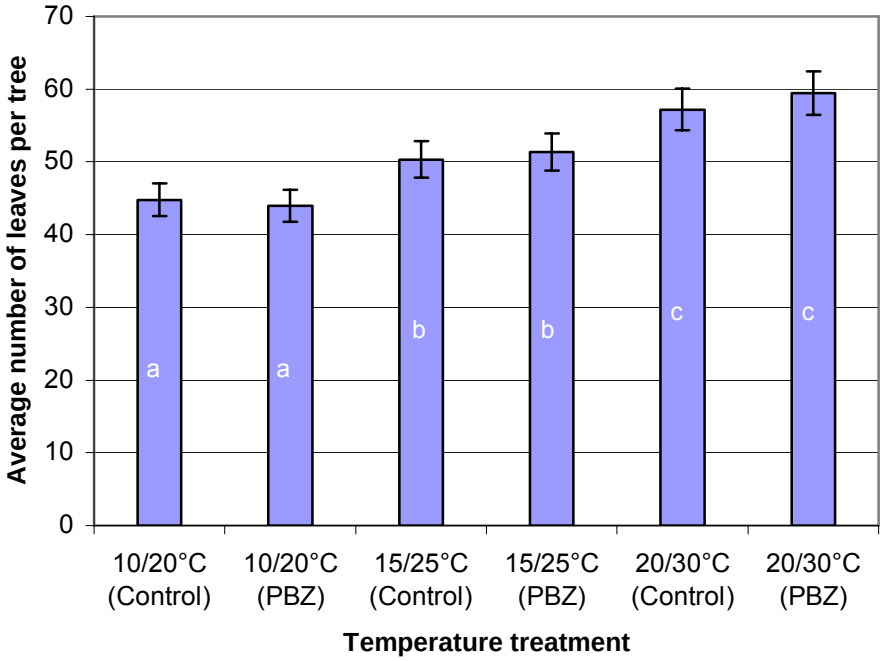

Figure 5.6 The average number of leaves produced by *Moringa oleifera* trees from both the PBZ and the control treatments during the 32-week period at the three temperature regimes. Treatment means with letters in common are not significantly different at $P \leq 0.05$.

The number of leaves formed during the trial period differed only marginally between the TRS, while the difference in leaf area between the three TRS was substantially more. Signifying that an increase in average leaf size, rather than the number of leaves was responsible for the difference in total leaf area amongst the three TRS.

The interaction effect between temperature, PBZ and hardening-off on net growth and average growth rates, for the entire trial period are given in Table 5.1 and 5.2 respectively. The parameters measured were tree height, stem diameter and leaf area increases.

Table 5.1 The effect paclobutrazol (PBZ) and hardening-off treatments had on tree growth across the three temperature regimes at the end of the 32-week trial period. The measured parameters were average tree height (cm), stem diameter (mm) and leaf area (cm^2). Different letters indicate significant differences at $P \leq 0.05$ according to the F-test.

		Treatment					
Nett growth parameter	**Temperature regime**	**PBZ (HO)**	**PBZ (NHO)**	**Control (HO)**	**Control (NHO)**	**PBZ* (Average)**	**Control* (Average)**
Tree height	**10/20°C**	21.9864[a]	8.4864[a]	42.7591	13.8545[a]	15.2364	28.3068
(cm)	**15/25°C**	104.3455[c]	72.1136[b]	86.7955[c]	60.3636[b]	88.2296	73.5796
	20/30°C	207.8909[d]	182.9500	208.2682[d]	169.4045	195.4205[g]	188.8364[g]
Stem diameter	**10/20°C**	5.2909[bc]	2.7045[a]	6.6409[c]	3.5136[ab]	3.9977	5.0773
(mm)	**15/25°C**	15.3364	10.9091[d]	10.5227	8.9182[d]	13.1228	9.7205
	20/30°C	25.9818[f]	24.4045[e]	23.2682[ef]	20.0591	25.1932	21.6637
Leaf area	**10/20°C**	749.9253[b]	329.3704[a]	969.2316[b]	195.4243[a]	539.6479[h]	582.3280[h]
(cm^2)	**15/25°C**	1829.5988[b]	1437.0988[c]	260.6392[a]	285.8426[a]	1633.3488	273.2409
	20/30°C	1743.3538[c]	1664.5941[c]	1481.3783[c]	1635.3324[c]	1703.9740[i]	1558.3554[i]

* Averages were subjected to separate statistical analysis

Table 5.2 The effect paclobutrazol (PBZ) and hardening-off treatments had on tree growth rates across the three temperature regimes for the 32-week trial period. The measured parameters were average growth rates of tree height (mm/week), stem diameter (mm/week) and leaf area (cm^2/week). Different letters indicate significant differences at $P \leq 0.05$ according to the F-test.

		Treatment					
Growth rate parameter	**Temperature regime**	**PBZ (HO)**	**PBZ (NHO)**	**Control (HO)**	**Control (NHO)**	**PBZ* (Average)**	**Control* (Average)**
Tree height	**10/20°C**	6.870739[a]	2.651989[a]	13.36222	4.329545[a]	4.7614	8.8459
(mm/week)	**15/25°C**	32.60795[c]	22.53551[b]	27.12358[c]	18.86364[b]	27.5717[g]	22.9936[g]
	20/30°C	64.96591[d]	57.17188	65.08381[d]	52.93892	61.0689[h]	59.0114[h]
Stem diameter	**10/20°C**	1.653409[bc]	0.84517[a]	2.075284[c]	1.098011[ab]	1.2493[g]	1.5866[g]
(mm/week)	**15/25°C**	4.792614	3.409091[d]	3.288352	2.786932[d]	4.1009	3.0376
	20/30°C	8.119318[f]	7.62642[e]	7.271307[ef]	6.268466	7.8729	6.7699
Leaf area	**10/20°C**	23.435165[b]	10.292824[a]	30.288489[b]	6.107009[a]	16.8640[g]	18.1977[g]
(cm^2/week)	**15/25°C**	57.174963[b]	44.909338[c]	8.144974[a]	8.932582[a]	51.0422	8.5388
	20/30°C	54.479807[c]	52.018565[c]	46.293071[c]	51.104136[c]	53.2492[h]	48.6986[h]

* Averages were subjected to separate statistical analysis

5.4.3 Paclobutrazol and temperature effect on total tree biomass

After trial termination the total fresh and dry tree mass (g) as well as the individual weight of the various tree components (roots, stem and canopy), were determined and are illustrated in Figure 5.7 and Figure 5.8 respectively. Following statistical analysis the differences in total tree weight between the three TRS were found to be significantly different, this was also the case for the stem and leaf mass (except between the 15/25°C and 20/30°C regime). The root mass (both fresh and dry) however did not differ significantly ($P \leq 0.05$) amongst all temperature and PBZ treatments.

While stem, leaf and total tree weight were consistent with former result and demonstrated an increase in weight (both fresh and dry) with the increase in TR, root weight on the other hand was seemingly unaffected. The trees expressed a root:shoot ratio of 0.2, 0.5 and 1.4 for the 10/20°C, 15/25°C and 20/30°C TRS respectively. Although the effect of the growing temperature on dry matter partitioning varies amongst plant species, the decrease in growing temperature generally leads to an decrease in the root:shoot ratio in plants (Raghavendra, 1991, Clarkson *et al.*, 1988). The increase in root:shoot ratio with the increase in TR is primarily due to an increase in shoot mass as the root mass essentially remained unchanged across all temperature and PBZ treatments.

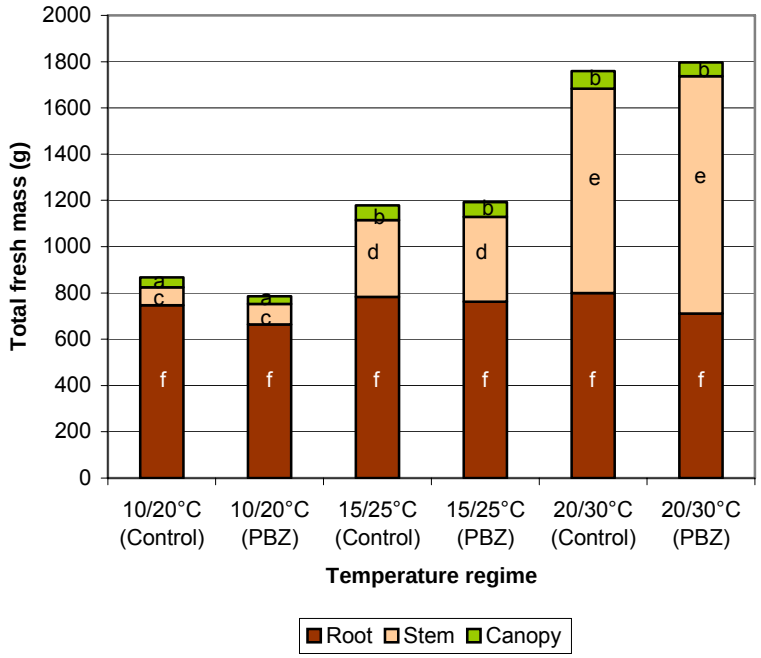

Figure 5.7 The total average tree fresh mass (g) and the fractional mass of the various tree components (roots, stem and canopy) as influenced by PBZ and three temperature regimes at trial termination. Treatment means with letters in common are not significantly different at $P \leq 0.05$.

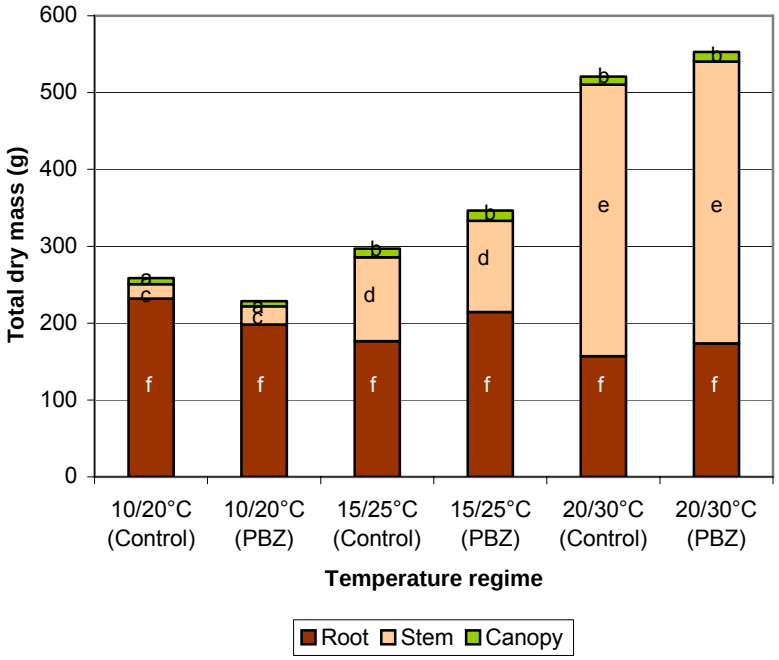

Figure 5.8 The total average tree dry mass (g) and the fractional mass of the various tree components (roots, stem and canopy) as influenced by PBZ and three temperature regimes at trial termination. Treatment means with letters in common are not significantly different at $P \leq 0.05$.

The consistency of root mass across a range of PBZ and TRS is uncommon and therefore not well documented, however the limited size of the bags cannot be excluded as a possible contributory factor to this phenomenon. However under optimal growing conditions trees maintain a certain root:shoot ratio, which changes once trees are subject to environmental stresses, one of which being sub-optimal temperatures. This is due to a readjustment of assimilates between the source and sink organs within trees (Ericsson *et al.*, 1996). However what these results might insinuate is that even under lower growing temperatures *M. oleifera* trees seemingly maintained carbohydrate partitioning to the roots at the expense of the shoots. Given that trees from the higher growing temperatures demonstrated superior growth despite all having a similar root mass/volume. However, while the root mass (both fresh and dry) did not differ significantly ($P \leq 0.05$) amongst the three TRS, the dry root mass illustrated a tendency towards reduced root mass with the increase in TR. This is indicative of lower root densities, which is likely the result of lower carbohydrate levels, depleted in the roots by increased shoot growth observed with the increase in temperature. Representative samples of roots from the three TRS are illustrated in Figure 5.9.

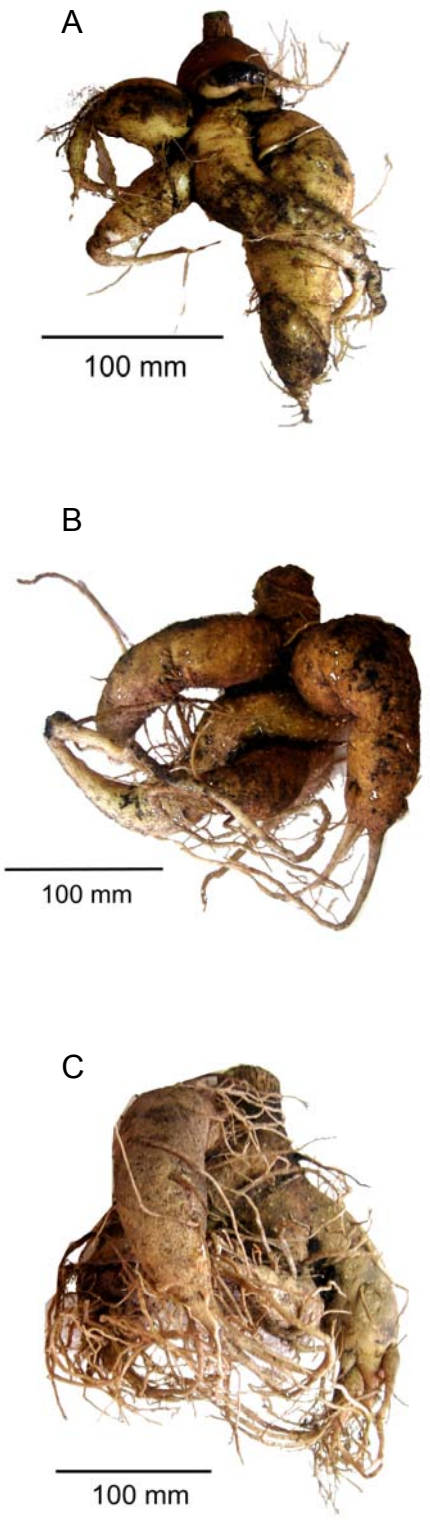

Figure 5.9 Tuberous *Moringa oleifera* roots from the various temperature regimes. A= 10/20°C, B= 15/25°C and C= 20/30°C.

Contrary to customary expectation, the application of the growth inhibitor PBZ did not uniformly reduce tree growth but rather expressed inconsistently throughout the three TRS. According to Basra (2000), *Hedera helix* L. is the only reported plant thus far, to have exhibited growth increases following a PBZ treatment during a study conducted by Horrell *et al*. (1990). The variation in growth across the three TRS, suggests that the efficacy of PBZ on *M. oleifera* is temperature related, stimulating growth at high- while reducing growth at low temperatures.

PBZ is a member of the triazole family. Triozoles, besides their growth regulatory properties also possess the ability to protect plants from various abiotic stresses and are therefore often referred to as multi-protectants (Manivannan, 2007; Jaleel, 2007). Sankar *et al.,* (2007) found PBZ to effectively reduce the negative effects of drought stress in groundnut (*Arachis hypogaea* L.) plants, by increasing antioxidant levels. Triazoles not only increase anti-oxidant concentrations but also enhance the activity of scavenging enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT). In addition, PBZ increases plant drought tolerance through numerous physiological changes such as decreasing transpiration through increased stomatal resistance, thickening of leaves, chlorophyll increases, enlarged chloroplasts and increases in the root:shoot ratio (Manivannan, 2007; Jaleel, 2007; Fernández, 2006). Despite PBZ primarily being used as a growth retardant, its ability to ameliorate drought and high temperature stresses in plants has been extensively studied and confirmed (Fletcher *et al.*, 2000).

These advantageous characteristics of PBZ are most probably the reasons for the unexpected growth increases observed in the trees treated with PBZ at both the

15/25°C and 20/30°C TRS. In Chapter 4, the main cause of the periodic leaf drop was attributed to temporary water stress as a result of the higher daytime temperatures. A closer look at Figure 5.5, revealed that the trees treated with PBZ at the 15/25°C and 20/30°C TRS, but more explicitly at the 15/25°C TR, maintained a higher leaf area, even though they were subject to the same environmental growing conditions. Growth at both the 15/25°C and 20/30°C TRS, were previously found to be the most affected by the higher temperatures and temporary water stress, however growth of the PBZ treated trees was superior to the non-treated trees under the same TRS. Thus the PBZ treated trees were seemingly less affected by then temporary water stress, confirming the attenuating effect of PBZ on drought stress in *M. oleifera*. The superior growth observed in the PBZ treated trees, is most probably due to the advantages of their increased drought tolerance, compared to their control treatment counterparts.

Reduced temperatures as a cause for increased leaf thickness, has extensively been studied, and confirmed by Higuchi, (1999), Trochoulias and Lahav, (1983) Lahav and Trochoulias, (1982) and Wiebel *et al.* (1994). The same phenomenon was observed in *M. oleifera* trees and comprehensively discussed in Chapter 4 under section 4.5.2.2. According to Jaleel (2007) similar leaf thickening, induced by a PBZ application was observed in *Catharanthus roseus* (L.). As both temperature and PBZ seemingly influence leaf thickness, the combined effect of these factors on leaf thickness of *M. oleifera* is illustrated in Figure 5.10. The PBZ treatment on the other hand did reveal slight, but non-significant ($P \leq 0.05$) increases in leaf thickness at both the 15/25°C and 20/30°C TRS. The growth increases observed in the PBZ treated trees can thus not exclusively be attributed to the variation in leaf thickness, due to

non-significant differences, however the mutual effect of leaf thickening and other physiological adaptations are most probably responsible for the growth increases.

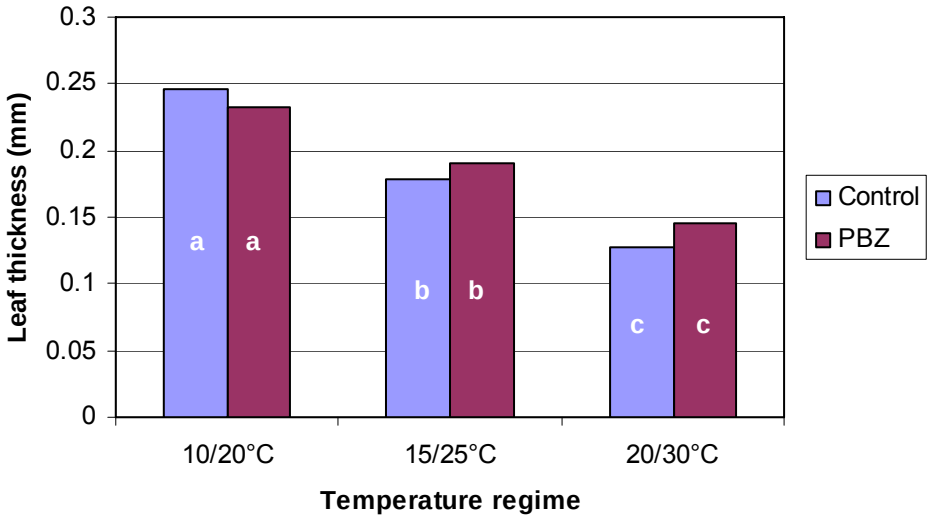

Figure 5.10 The effect paclobutrazol (PBZ) on leaf thickness of *Moringa oleifera* at three different temperature regimes. Different letters indicate significant differences at $P \leq 0.05$ according to the F-test.

5.5 Conclusion

The use and efficacy of PBZ as a growth-retardant on *M. oleifera* is temperature dependant. The sub-optimal growing conditions prevalent at the low 10/20°C TR caused a considerable reduction in growth; that was further exacerbated by the application of PBZ. Despite the effectiveness of PBZ as growth retardant at low temperatures, its use is not advisable as plants are severely stressed and growth virtually ceases. The expected growth retardation following the PBZ application was not observed at both the higher 15/25°C and 20/30°C TRS, but surprisingly increased tree growth. Despite significant differences in both fresh and dry mass between the three TRS, PBZ had seemingly no effect on the total biomass production of trees within the different temperature treatment regimes. PBZ is also known to act as a plant protector against several environmental stresses. The

protection offered by PBZ against such stresses, outweighed the reduction in growth, resulting in growth increases with the increase in temperature. The application of PBZ may, therefore, be effective as a stress-alleviator in areas of high temperatures and low rainfall, to ensure acceptable growth, despite being exposed to adverse growing conditions.

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CHAPTER 6

FLOWERING OF *MORINGA OLEIFERA* LAM. AT THREE TEMPERATURE REGIMES.

6.1 Summary

Temperature is known to be a floral initiator in numerous plants, for this reason *Moringa oleifera* trees were exposed to three temperature regimes (TRS), while their flowering response was documented. Both the highest instances of flowering trees (87.5%) as well as the highest pollen viability (82.7% ± 2.7%) were measured at the intermediate 15/25°C temperature regime (TR). The prevalence of inflorescence reversion at the 20/30°C regime suggests that subsequent to floral induction, trees should indeed be able to flower at the 20/30°C TR. Whereas the 10/20°C TR probably did favour floral induction, the generally low temperatures however hindered flower initiation.

6.2 Introduction

Flowering is a central part in the life cycle of all angiosperms as it assures the preservation of the species through seed formation (Tan and Swain, 2006). Given that the fruit/seed yield is a direct function of floral induction, plants have evolved mechanisms to ensure that flowering occurs at the appropriate time, guaranteeing reproductive success. While environmental stimuli such as photoperiod, temperature and water availability are the main factors responsible for flower induction, endogenous physiological signals such as growth status, plant size/age, hormones

and nutrient flow, are secondary factors that ensure floral initiation at the right time (Ainsworth, 2006; Bernier *et al.*, 1993; Levy and Dean, 1998).

As the flowering of one-year-old *M. oleifera* trees was observed under field conditions, the juvenility of the trees should not influence flowering. Subjecting *M. oleifera* trees to different TRS, should reveal whether their flower initiation is in fact temperature-regulated. The aim of this study was thus not only to determine the effect of temperature on flowering but also its effect on pollen viability through pollen viability testing. Both these parameters are influential to successful fruit/seed production. According to Bhattacharya and Mandal (2004) each *M. oleifera* flower produces on average 23525 pollen grains with delayed stigma receptivity to promote cross pollination, whilst fruit set was observed in 10.3% of flowers.

6.3 Materials and Methods

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

Mature, two year old *M. oleifera* trees growing in 5 liter black plastic bags under shade netting at the University of Pretoria's Experimental Farm were used throughout this flowering trial. The trees were cultivated from *M. oleifera* seed sourced from Malawi. Prior to the placement of 8 trees into each of the three temperature treatment regimes (10/20°C, 15/25°C and 20/30°C), they were transplanted into 10 liter black plastic bags, filled with a commercial bark potting

medium manufactured by Braaks (Pty) Ltd. Trees at three fluctuating night/day TRS were exposed to natural daylight, while temperature fluctuations of ± 2°C were permissible due to influx radiation. The average measured PAR at 12:00 on a cloudless day inside the temperature-regulated greenhouses was 1350 $\mu mol.m^{-2}.s^{-1}$. Sixteen weeks after trial initiation, a single liquid fertilizer application of 20g LAN (28) per tree was made to adjust nitrogen deficiencies evident from soil analysis results (Appendix, Table 8.A2). All trees received manual irrigation three times a week until field-capacity was reached, as the excess water was able to drain from bags.

The eight mature, two year old trees within each TR were monitored for flowering throughout the 32-week trial period whilst the growth trials of the other 264 trees were taking place. The effect temperature had on flowering was recorded by merely counting the number of trees that flowered throughout the 32-week trial period within each of the three TRS.

Pollen viability test

The *in vitro* pollen germination test was performed using the hanging drop method (Shivanna and Rangaswamy 1992). According to Bhattacharya and Mandal (2004) a 10% sucrose solution with a 200 µg/ml boric acid (H_3BO_3) concentration had the highest pollen germination percentages for *M. oleifera*. For this reason the above mentioned concentration was used for the pollen germination trials across all three TRS. Pollen collected from freshly opened flowers at the various TRS was immersed into a drop of the sucrose solution using a needle. Pollen was left to germinate at room temperature (20°C) for 2 hours. After which the cover slip containing the pollen

was placed onto a microscope slide and observed under a Leitz Biomed light microscope, while digital pictures were taken with an Olympus Camedia C-4000 Zoom camera. Hundred randomly selected pollen grains per slide were assessed for their viability. Pollen was considered viable once the pollen tube length was equal or greater than the diameter of the individual pollen grains.

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) to enable the comparison between treatment means.

6.4 Results and Discussion

The effect of the different TRS on flowering and pollen viability are illustrated in Table 6.1. Both the highest instances of flowering and pollen viability were found at the 15/25°C TR. Differences between the 10/20°C and 15/25°C as well as the 15/25°C and 20/30°C regime were found to differ significantly from one another, while measurements from the 10/20°C and 20/30°C regimes did not.

Table 6.1 Temperature effect on tree flowering as well as *in vitro* pollen germination percentages of *Moringa oleifera*. Different letters indicate significant differences at $P \leq 0.05$ according to the F-test.

	Temperature regime		
	10/20°C	**15/25°C**	**20/30°C**
Proportion flowering trees (%)	25.0[a]	87.5[b]	12.5[a]
Pollen viability (% ± SD)	68.7 ± 3.2[a]	82.7 ± 2.7[b]	70.4 ± 3.8[a]

From Table 6.1 it is apparent that the 15/25°C TR favours flowering (Figure 6.1), whereas both the other regimes appear non-conducive to flowering.

Figure 6.1 Inflorescence initiation of *Moringa oleifera* at the 15/25°C treatment regime.

Upon further investigation, it was found that trees at the high 20/30°C TR were subject to inflorescence reversion (Figure 6.2). This reversion from reproductive to vegetative growth is common amongst numerous plant species and generally the result of environmental circumstances, where the flowering is induced but flower initiation does not transpire. Instead the floral meristem reverts to vegetative growth, by producing leaves (Battey and Lyndon, 1990). The poor flower initiation observed

at the 20/30°C regime suggests the necessity of vernalization to enable flower initiation in *M. oleifera* trees. Based on the high percentage flowering trees at the 15/25°C regime, a mere 5°C reduction in minimum temperature from 20°C to 15°C seems to be sufficient for overcoming the floral induction/inhibition. Profuse flowering was reported at high night/day temperatures (20/30°C) by Mathew and Rajamony (2004), providing evidence that the high temperatures alone are not detrimental to floral development. However these field-grown trees were most probably exposed to lower temperatures (<15°C) that induced flowering. The floral reversion observed in trees at the unvarying 20/30°C regimes, suggest that following induction, trees would indeed flower at this TR. Despite the low 10/20°C regime probably being effective to induce flowering, temperatures were too low to initiate flowering. The comparably low number of flowering trees at the 10/20°C regime is however as a result of growing temperatures being persistently low. For commercial seed/oil production purposes, this TR is thus unsuitable, given the meager flowering.

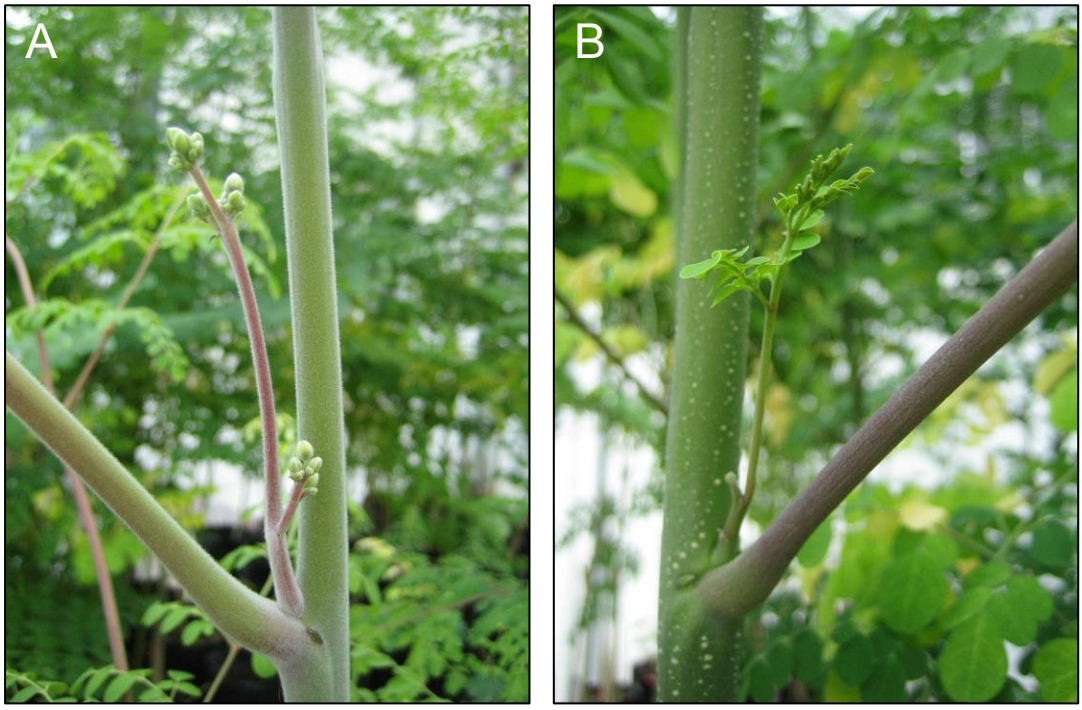

Figure 6.2 A – Normal inflorescence development of *Moringa oleifera* at the 15/25°C regime. B – An instance of induction reversion witnessed at the 20/30°C regime.

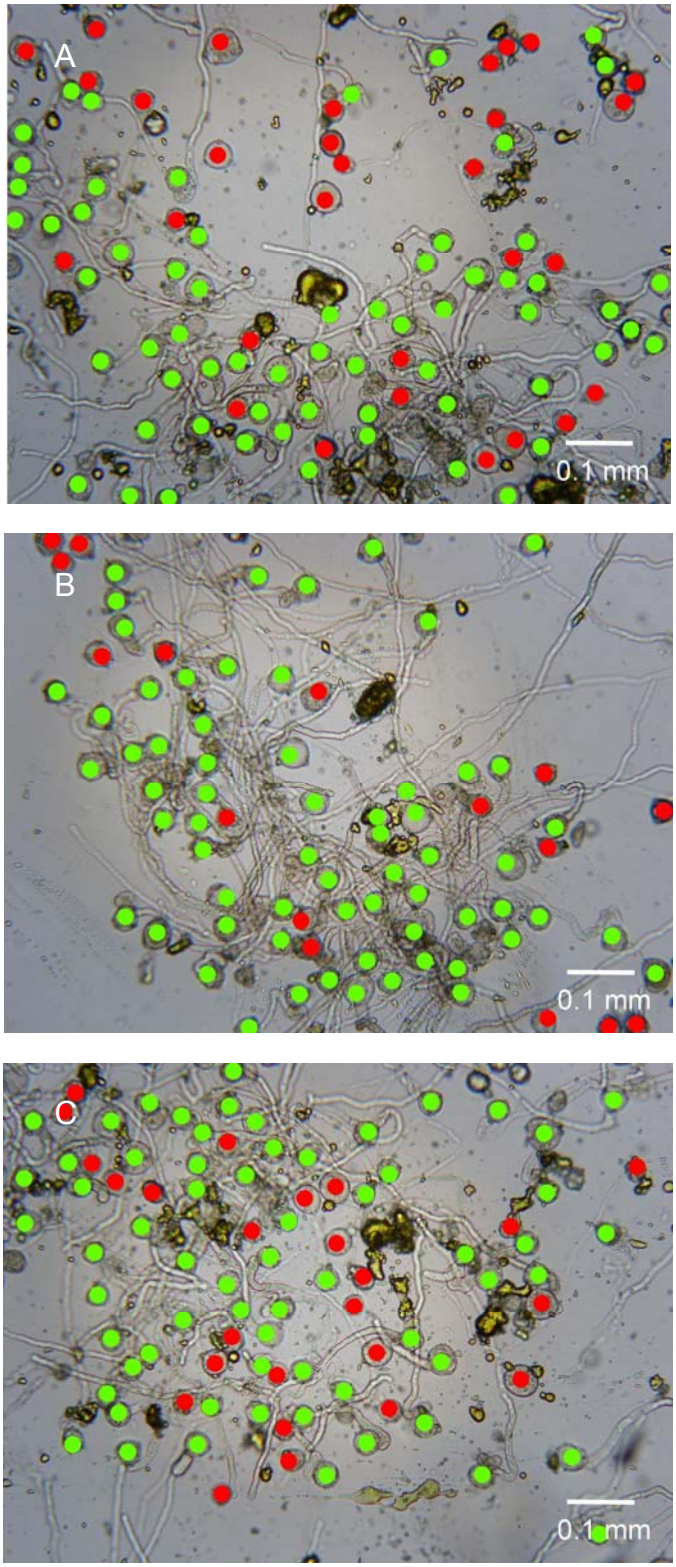

Figure 6.3 *In vitro* pollen germination of *Moringa oleifera.* Green spots are indicative of viable/germinated pollen grains, while red spots indicate non-viable/non-germinated pollen grains. A = 10/20°C, B = 15/25°C and C = 20/30°C.

Variation in pollen viability across the three TRS is illustrated in Figure 6.3 as well as in Table 6.1. The pollen germination percentage at the 15/25°C regime was the highest and significantly higher than both the higher 20/30°C and lower 10/20°C TR. Mathew and Rajamony (2004) and Bhattacharya and Mandal (2004) found the pollen viability of trees growing throughout India to be 88% and 82% respectively, this is similar to the pollen viability of 82.7% measured at the 15/25°C TR. All of the above pollen viability results were obtained using a sucrose concentration of 10%. Temperature and relative humidity (RH) are the main environmental factors affecting pollen viability. Decreases in both of these factors in the growing environment generally improve pollen viability (Shivanna and Rangaswamy, 1992), for *M. oleifera* however pollen viability was lowest at the 10/20°C regime. Previous research has also shown unfavourably high (Prasad *et al.* 2006) and/or low (Tuinstra and Wedel, 2000) growing temperatures to considerably reduce pollen viability. This would elucidate the superior flowering and pollen viability at the moderate 15/25°C regime. Future research might have to consider comprehensive pollen viability testing at a greater range of TRS, and its consequent effect on fertilization and seed development

6.5 Conclusion

Amongst the tested TRS, 15/25°C was found the most favourable regime for both flowering and pollen viability. However due to the absence of inflorescence induction observed at the higher 20/30°C regime, flowering should occur once these trees had been vernalized. The low 10/20°C TR was found non-conducive to flowering as floral initiation was limited to merely 25% of the trees.

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CHAPTER 7

GENERAL DISCUSSION

Moringa oleifera Lam. is a versatile plant with numerous benefits. One such benefit, and also the motivation for this research is the oil, which once extracted from the seed, can be used to produce bio-diesel (Ramachandran *et al.*, 1980; Jahn, 1988; Rashid *et al.,* 2008). The production of its oil as well as the exploitation of other beneficial products on an industrial scale, would require the establishment of commercial *M. oleifera* plantations. However, successful plantation establishment and the subsequent management thereof, require comprehensive cultivation guidelines. Specific cultivation and management guidelines for *M. oleifera* are relatively limited due to inadequate research conducted in this field to date (National Research Council, 2006). Temperature is an important environmental factor which affects the tree growth and distribution especially due to its preference towards tropical climates (Sakai and Larcher, 1987; Grace, 1988). Thus, the main aim of this research was to provide basic guidelines on the temperature requirements for *M. oleifera* trees. Following the evaluation of tree growth from germination to flowering across three chosen temperature regimes (TRS), the objectives of this study have been met, providing valuable insight into the response of *M. oleifera* to temperature.

Seed germination results revealed the highest germination rate and uniformity at the 20/30°C temperature regime (TR), making it seemingly the best TR for commercial seedling production as high germination rates, with acceptable uniformity are desirable. The increase in temperature however induced seed dormancy, reducing

germination percentages. An incubation period at a lower temperature of 10-20°C for 5-7 days should break this dormancy, which would improve seed germination percentages. Seed sown directly into the field, would most certainly experience greater temperature extremes, where low night temperatures encourage higher germination percentages while higher daytime temperatures will promote shorter MGT, higher germination uniformity and growth. Trees would also benefit from the subsequent hardening-off effect presented by field conditions which lead to improved post seedling stage growth, as discussed in Chapter 4.

Results from Chapter 4 demonstrated how tree growth was affected by the change in TR. The hardening-off of trees prior to their introduction to the three TRS proved to be greatly advantageous, since the hardened-off trees surpassed the non-hardened trees across all measured parameters at all three TRS. Despite the high 20/30°C TR being the most beneficial for seedling cultivation (Chapter 3), the hardening-off of seedlings is highly recommend, particularly if seedlings are to be planted in a medium (15-25°C) to low (10-20°C) temperature environment.

Tree height and stem thickening increased proportionally with the increase in TR. The proportional increases of both primary (stem length) and secondary (stem diameter) growth in response to the three TRS, demonstrate the correlation between the apical and lateral meristems. Cell division and elongation taking place at the apical meristem are thus interrelated to the cell division at the lateral meristem, regardless of the growing temperature. On the other hand leaf thickness decreased with the increase in temperature. Previous research has shown that plant adaptations in response to their environmental conditions are often expressed

through anatomical modifications (Shao *et al*., 2008). Leaves at the 10/20°C regime were on average approximately twice a thick as those from the 20/30°C treatment. A broader spongy mesophyll layer was found responsible for the change in leaf thickness, this is a anatomical adaptation to minimize photoinhibition, a common hindrance particularly at low temperatures. According to Terashima, *et al.*, (2001) advantages of thicker leaves is the larger intracellular chloroplast surface area with a potentially higher CO_2 assimilation rate, however the increase in leaf thickness also increases the bulk resistance to CO_2 inside the leaf.

With the increase in temperature, the increase in leaf area became less linear as a result of frequent cycles of regular leaf drop followed by renewed flushes (Figure 4.5, page 54). These cycles being the result of the increased transpirational water loss at higher temperatures as the moisture demands of the increased leaf areas exceed the supply by the roots as discussed in Chapter 4 (page 66). The number of leaves formed throughout the trial period did not differ to the same extent to which the number of leaflets per leaf did between the TRS, since the leaves formed at the higher TRS were larger as a result of more leaflets per leaf. Consequently the fluctuations in leaf area were also more severe at the higher TRS given that leaves had a significantly higher leaf area, and a loss of a single leaf would reduce the leaf area to a greater extent at the 20/30°C TR than those of the 10/20°C TR. This combined with the increased transpirational water loss at the higher TRS resulted in the aggravated leaf area fluctuations with the increase in TR.

Chapter 5 explored the probability of inducing flowering through the application of a plant growth regulator (PGR). While the PGR paclobutrazol (PBZ) is known and

generally used as a growth retardant and floral inducer in tree crops, the only reduction in growth was observed at the low 10/20°C TR, whilst at both the higher TRS growth was promoted. Proving that, the effect PBZ had on *M. oleifera* trees was temperature dependant. Flowering in contrast, remained unaffected by PBZ as no differences were observed between the PBZ and control treatments. PBZ has been identified as a plant protector against several environmental stresses (Fletcher *et al.*, 2000). The unexpected growth increases at the higher TRS, were thus as a result of protection the treated trees received against the damaging effects of high temperatures and water stress. Utilizing PBZ as a stress-alleviator at moderate concentrations in unfavorable growing conditions of high temperatures and possibly even low rainfall, would ensure superior growth compared to their non treated counterparts. Although PBZ has successfully reduced growth at low temperatures, the use thereof however under these conditions is not advisable as plants were severely stressed and growth virtually ceased. Given that only a single PBZ concentration of 100 mg.liter^{-1} (active ingredient) was tested throughout this trial, the effect of various concentrations is unknown. Future trials should possibly look into the effect of a wider concentration range at various temperatures regimes, to establish the optimal PBZ concentration at a given temperature for maximum stress alleviation.

Flowering was also found to be significantly affected by temperature with the 15/25°C TR being the most conducive to both flowering and pollen viability. Trees at the 20/30°C TR demonstrated no inflorescence induction, suggesting that flowering would only take place once these trees had been exposed to lower temperatures. Fortunately under most field conditions trees will be exposed to temperatures below

20°C (even if only momentarily), ensuring inflorescence induction. Yet consistently low growing environments (10-20°C) are non conducive to flowering seeing as merely 25% of the trees flowered at the 10/20°C TR.

Based on the insight gained with regards to the temperature effect on *M. oleifera* from the preceding sections, Figure 7.1 can be used to identify prospective production areas.

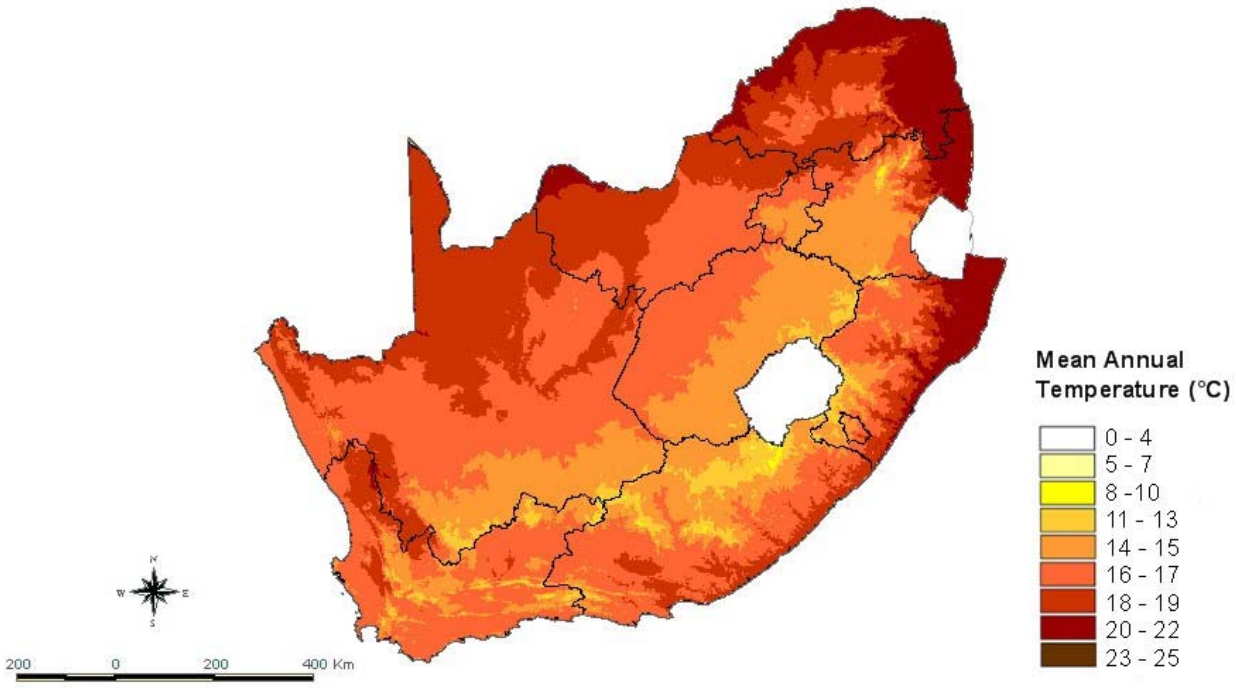

Figure 7.1 Mean Annual Temperature of South Africa.
http://0-www.environment.gov.za.innopac.up.ac.za/enviro-info/sote/nsoer/general/about.htm

The most favourable TR with the exception of flowering, but with regards to overall tree growth, with the highest values measured across all parameters was the 20/30°C regime, confirming the preference of *M. oleifera* trees for tropical growing environments. Cultivation of *M. oleifera* under tropical environments in South Africa would restrict production to the coastal regions of Kwa-Zulu Natal. Given that an average daytime growing temperature of >20°C for the germination and cultivation is preferable, satisfactory growth during the hot summer months in sub-tropical climates ought to be achievable, provided the winters are mild as trees are frost tender. Production should therefore be feasible in areas identified to have an annual average temperature of 18-19°C (Figure 7.1) and higher, such as the Kwa-Zulu Natal Midlands, North-eastern Mpumalanga, Limpopo, northern Gauteng, North West, Northern Cape and coastal regions of the Eastern Cape. Since lower growing temperatures (15-25°C) result in thicker leaves, increased flowering and pollen viability (as observed in the 10/20°C TR), trees might in fact benefit from the greater temperature variation of sub-tropical environments, compared to a constant tropical climate. The greater Free-state and Western Cape Provinces however would generally be unsuitable for the production of *M. oleifera* based on the mean annual temperatures from Figure 7.1. Not only would tree growth be slow and prone to suffer frost damage, but flowering would also be too meager to justify their cultivation in these areas. As these trials were conducted under controlled environmental conditions, similar growth trials should possibly be repeated under field conditions to substantiate these results.

While the temperature trial performed during this research have yielded valuable insight into the climatic requirements of *M. oleifera* trees, areas that require

additional insight through follow up research have been identified. Additional, more detailed temperature trials investigating the temperature effect on flowering prolificacy and frequency would also be beneficial. Other production aspects succeeding flowering that are also affected by temperature such fruit set, development and yield probably need further investigation to enable more comprehensive production guidelines. While the effect of various climatic conditions on the development of lipid bodies in the seed, would provide insight into the affectability of oil formation by different climates.

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APPENDIX

Table 8.A1 Plant analysis results

Table 8.A2 Soil analysis results

Table 8.A3 Weather data

As a result of limited space, the complete statistical analysis of all data as analyzed by the Department of Statistics at the University of Pretoria has not been included in this dissertation, it can however be made available on request.

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PLANT ANALYSIS:

28 February 2008

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DatumOntvang: 2008/02/19

Taak /Task No: **255**

Jaar	LabNo	VeldNo	N	P	Ca	K	Mg	Na	S	Cu	Fe	Mn	Zn	B
Year		FieldNo	%	%	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
2008	159	Q1	3.15	0.69	2.03	1.02	0.58	0.01	1.08	6	107	51	38	0.0
2008	160	Q2	2.70	0.56	2.06	0.75	0.54	0.00	0.88	6	81	57	35	0.0
2008	161	Q3	2.30	0.53	1.67	0.91	0.55	0.01	0.91	5	147	47	44	0.0
2008	162	Q4	2.06	0.47	1.97	0.78	0.54	0.01	0.87	5	123	50	30	0.0
2008	163	Q5	2.73	0.57	1.73	1.06	0.71	0.06	1.02	6	206	53	42	0.0
2008	164	Q6	2.64	0.51	1.64	0.95	0.60	0.10	1.07	6	114	38	24	0.0

Table 8.A2 Soil analysis results

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					Ammonium Acetaat Oplosbaar:					
					Ammonium Acetate Extractable:					
Jaar	**Lab No**	**Veld No**	**pH water**	**P Bray I**	**Ca**	**K**	**Mg**	**Na**	**NH4**	**NO3**
Year		**Field No**		**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**
2007	3179	Moringa Groeimedium A	6.8	232.2	2284	138	527	89	54	1500
2007	3180	Moringa Groeimedium B	6.7	260.4	2350	180	576	99	22	218

Table 8.A3 Weather data

Weather data measured for the entire 32-week trial period at 12:00 AM.

Year	Day Of Year	Trial week	Time Of Day	Average Temperature (°C) (outside)	Average Relative Humidity (%) (outside)	Wind speed (m/s) (outside)	Radiation ($W.m^{-2}$) (outside)	Radiation ($W.m^{-2}$) (inside)
2007	241	1	12:00	18.38	52.09	3.59	991	776
2007	242	1	12:00	23.01	39.5	3.61	951	744
2007	243	1	12:00	24.45	41.67	4.291	901	705
2007	244	1	12:00	27.07	17.68	3.03	983	769
2007	245	1	12:00	26.59	24.64	2.621	1010	791
2007	246	1	12:00	26.48	28.11	3.448	986	772
2007	247	1	12:00	25.01	37.86	3.787	971	760
2007	248	2	12:00	27.79	23.83	3.323	977	765
2007	249	2	12:00	27.93	20.76	3.062	1005	787
2007	250	2	12:00	22.8	51.88	3.407	972	761
2007	251	2	12:00	21.48	59.34	3.133	1017	796
2007	252	2	12:00	26.51	37.92	4.109	980	767
2007	253	2	12:00	27.05	27.4	2.13	946	740
2007	254	2	12:00	25.79	36.83	4.003	1012	792
2007	255	3	12:00	28.84	25.47	3.844	1019	798
2007	256	3	12:00	28.15	30.8	3.853	1014	794
2007	257	3	12:00	26.46	27.41	3.975	1027	804
2007	258	3	12:00	22.86	54.36	3.722	1021	799
2007	259	3	12:00	26.09	39.27	3.956	1028	805
2007	260	3	12:00	22.59	58.78	4.089	1027	804
2007	261	3	12:00	22.46	57.36	2.715	1045	818
2007	262	4	12:00	23.72	54.6	2.351	1025	802
2007	263	4	12:00	24.7	46.05	2.218	1070	838
2007	264	4	12:00	26.31	29.37	3.335	1117	874
2007	265	4	12:00	28.07	19.81	4.744	1109	868
2007	266	4	12:00	29.78	15.36	5.183	1120	877
2007	267	4	12:00	27.7	15.89	4.24	1125	881
2007	268	4	12:00	20.78	66.35	3.221	976	764
2007	269	5	12:00	17.28	79	3.38	468.6	367
2007	270	5	12:00	15.09	96.8	2.652	139.5	109
2007	271	5	12:00	16.59	89.4	1.44	211.5	166
2007	272	5	12:00	21.06	75.3	3.548	1071	838
2007	273	5	12:00	21.08	77.3	1.945	604.3	473

2007	274	5	12:00	17.86	98.4	0.722	214.8	168
2007	275	5	12:00	17.65	97.9	2.787	662.7	519
2007	276	6	12:00	21.13	77.9	3.901	1081	846
2007	277	6	12:00	23.4	66.1	2.655	1009	790
2007	278	6	12:00	24.44	66.45	3.089	841	658
2007	279	6	12:00	17.44	100	1.37	430.9	337
2007	280	6	12:00	21.83	88.4	3.993	911	713
2007	281	6	12:00	26.73	64.55	2.698	952	745
2007	282	6	12:00	12.98	100	2.963	31.15	24
2007	283	7	12:00	20.06	64.16	1.76	726	568
2007	284	7	12:00	14.35	99.9	0.769	55.95	44
2007	285	7	12:00	18.91	82.5	2.62	1048	820
2007	286	7	12:00	24.13	60.26	1.938	921	721
2007	287	7	12:00	22.85	73.2	4.142	1090	853
2007	288	7	12:00	26.28	49.47	2.673	1059	829
2007	289	7	12:00	22.99	80.5	2.52	992	777
2007	290	8	12:00	23.98	69.57	2.493	1203	942
2007	291	8	12:00	26.26	54.19	2.553	1074	841
2007	292	8	12:00	20.87	85.2	2.781	535.2	419
2007	293	8	12:00	18.42	92.1	2.256	622.7	487
2007	294	8	12:00	21.24	77.9	2.221	1068	836
2007	295	8	12:00	22.75	58.02	2.42	1112	870
2007	296	8	12:00	23.78	46.66	4.244	1095	857
2007	297	9	12:00	25.21	40.15	2.592	1137	890
2007	298	9	12:00	17.95	84.1	2.867	111.8	88
2007	299	9	12:00	11.83	100	1.247	150.8	118
2007	300	9	12:00	12.97	100	1.97	182.5	143
2007	301	9	12:00	21.3	76.2	2.008	1049	821
2007	302	9	12:00	22.1	75.7	3.028	872	683
2007	303	9	12:00	24.58	67.81	2.144	1127	882
2007	304	10	12:00	20.99	85.1	2.201	468.2	366
2007	305	10	12:00	21.02	91.2	2.702	612.7	480
2007	306	10	12:00	24.86	68.89	2.627	789	618
2007	307	10	12:00	23.99	65.38	3.544	665.6	521
2007	308	10	12:00	26.86	58.33	3.465	1154	903
2007	309	10	12:00	26.42	61.25	4.942	1155	904
2007	310	10	12:00	25.29	71.1	3.545	776	607
2007	311	11	12:00	20.77	87.8	4.25	651	510
2007	312	11	12:00	21.91	71.9	1.641	303.3	237
2007	313	11	12:00	25.17	52.36	2.132	1125	881
2007	314	11	12:00	26.4	25.25	3.752	1231	964
2007	315	11	12:00	22.53	73.6	2.796	1048	820
2007	316	11	12:00	25.35	50.5	4.415	1211	948
2007	317	11	12:00	27.29	43.85	4.535	1194	935
2007	318	12	12:00	28.92	40.35	3.502	1216	952
2007	319	12	12:00	30.55	31.75	3.887	1216	952
2007	320	12	12:00	26.91	55.84	3.056	1188	930
2007	321	12	12:00	25.75	62.78	3.835	1180	924
2007	322	12	12:00	22.85	81	3.312	973	762
2007	323	12	12:00	25.04	72.4	4.957	1194	935
2007	324	12	12:00	26.92	59.28	3.418	804	629
2007	325	13	12:00	21.89	89.8	3.217	656.9	514

2007	326	13	12:00	26.06	65.28	5.493	803	629
2007	327	13	12:00	24.81	76.5	2.934	777	608
2007	328	13	12:00	21.13	99.9	1.709	511	400
2007	329	13	12:00	23.83	80.8	2.335	1054	825
2007	330	13	12:00	18.65	100	4.304	245.8	192
2007	331	13	12:00	25.65	63.96	4.53	1208	946
2007	332	14	12:00	17.95	88.6	2.315	347.9	272
2007	333	14	12:00	16.72	98	2.019	238.5	187
2007	334	14	12:00	17.53	100	1.23	231.3	181
2007	335	14	12:00	22.83	77.3	3.812	977	765
2007	336	14	12:00	24.69	79.7	2.991	998	781
2007	337	14	12:00	20.71	91.4	3.304	625.2	489
2007	338	14	12:00	23.13	80.2	3.018	816	639
2007	339	15	12:00	22.45	80.6	2.861	911	713
2007	340	15	12:00	25.71	70.6	2.701	1127	882
2007	341	15	12:00	17.71	100	2.135	479.8	376
2007	342	15	12:00	20.98	88.7	2.01	559	438
2007	343	15	12:00	20.59	89.5	2.532	960	751
2007	344	15	12:00	23.99	77.3	1.699	1094	856
2007	345	15	12:00	27.25	66.9	1.941	1173	918
2007	346	16	12:00	24.76	76.9	1.746	942	737
2007	347	16	12:00	24.55	75.8	2.25	1231	964
2007	348	16	12:00	24.65	79.5	3.158	932	730
2007	349	16	12:00	25.09	82.6	1.816	751	588
2007	350	16	12:00	24.43	88.8	2.751	634.7	497
2007	351	16	12:00	22.2	97.7	2.731	657.6	515
2007	352	16	12:00	22.35	73.4	6.442	1038	813
2007	353	17	12:00	18.64	72.5	4	1200	939
2007	354	17	12:00	18.59	73.5	2.386	748	586
2007	355	17	12:00	25.02	54.31	2.722	1234	966
2007	356	17	12:00	27.06	57.01	2.701	1189	931
2007	357	17	12:00	25.04	65.48	1.807	1209	946
2007	358	17	12:00	25.76	67.3	2.949	1201	940
2007	359	17	12:00	23.17	68.45	2.891	1218	953
2007	360	18	12:00	24.21	78.9	3.963	399.8	313
2007	361	18	12:00	26.29	71.2	2.388	1320	1033
2007	362	18	12:00	22.46	91.4	1.57	688.1	539
2007	363	18	12:00	24.68	51.07	6.378	1211	948
2007	364	18	12:00	24.11	35.25	3.614	1276	999
2007	365	18	12:00	24.12	60.6	2.13	1237	968
2008	1	18	12:00	26.49	54.95	2.569	1231	964
2008	2	19	12:00	26.72	59.94	2.946	1220	955
2008	3	19	12:00	28.78	58.12	3.06	1163	910
2008	4	19	12:00	18.06	100	1.221	105.1	82
2008	5	19	12:00	23.92	86.4	3.341	858	672
2008	6	19	12:00	24.13	84.8	2.761	645.1	505
2008	7	19	12:00	25.41	84.2	3.838	1026	803
2008	8	19	12:00	25.71	79.3	2.392	958	750
2008	9	20	12:00	15.44	100	3.567	93.1	73
2008	10	20	12:00	15.07	100	2.008	144.6	113
2008	11	20	12:00	15.98	100	1	256.5	201
2008	12	20	12:00	21.12	93.6	0.976	508	398

2008	13	20	12:00	24.66	83.6	1.996	892	698
2008	14	20	12:00	26.08	80.4	2.437	902	706
2008	15	20	12:00	25.22	84.3	3.261	843	660
2008	16	21	12:00	27.57	69.16	2.693	919	719
2008	17	21	12:00	25.72	80.6	2.444	832	651
2008	18	21	12:00	23.71	82.8	3.258	1161	909
2008	19	21	12:00	25.87	65.6	1.843	1166	913
2008	20	21	12:00	24.11	67.58	2.84	896	701
2008	21	21	12:00	23.49	64.92	2.373	1191	932
2008	22	21	12:00	24.37	69.11	2.661	1192	933
2008	23	22	12:00	28.58	58.82	2.031	688.1	539
2008	24	22	12:00	28.11	39.4	2.861	1184	927
2008	25	22	12:00	24.26	72.3	2.003	831	650
2008	26	22	12:00	25.75	63.58	2.507	1192	933
2008	27	22	12:00	27.93	63.11	2.629	917	718
2008	28	22	12:00	25.14	74.2	2.701	482.7	378
2008	29	22	12:00	26.49	41.95	2.797	1219	954
2008	30	23	12:00	27.41	54.46	2.84	1158	906
2008	31	23	12:00	23.96	73	1.388	524.4	410
2008	32	23	12:00	27.23	62.79	2.46	980	767
2008	33	23	12:00	28.05	51.95	2.812	838	656
2008	34	23	12:00	25.15	77.5	2.255	1137	890
2008	35	23	12:00	28.89	54.78	3.579	1124	880
2008	36	23	12:00	27.05	74.5	3.713	1098	859
2008	37	24	12:00	27.95	60.92	4.357	1142	894
2008	38	24	12:00	23.23	81.8	2.461	519.2	406
2008	39	24	12:00	26.35	71.9	2.603	1034	809
2008	40	24	12:00	27.33	73.7	2.631	1107	867
2008	41	24	12:00	27.43	77	2.96	1049	821
2008	42	24	12:00	23.61	54.6	3.871	1170	916
2008	43	24	12:00	23.65	41.91	2.693	1161	909
2008	44	25	12:00	23.83	30.1	3.009	1192	933
2008	45	25	12:00	24.63	60.71	2.578	1150	900
2008	46	25	12:00	25.76	67.56	3.297	1154	903
2008	47	25	12:00	28.54	49.89	3.522	1149	899
2008	48	25	12:00	26.13	72.4	2.845	1070	838
2008	49	25	12:00	27.92	56.26	1.748	1126	881
2008	50	25	12:00	30.33	27.28	4.444	1172	917
2008	51	26	12:00	30.34	19.38	3.379	1201	940
2008	52	26	12:00	30.82	29.68	2.128	1143	895
2008	53	26	12:00	31.09	32.27	2.177	1128	883
2008	54	26	12:00	27.68	56.68	3.704	1081	846
2008	55	26	12:00	28.08	54.8	2.786	1141	893
2008	56	26	12:00	28.39	40.47	4.194	1182	925
2008	57	26	12:00	27.02	64.39	4.851	1017	796
2008	58	27	12:00	26.14	58.3	1.964	1135	888
2008	59	27	12:00	27.66	54.08	1.98	1096	858
2008	60	27	12:00	27.81	46.16	2.301	1104	864
2008	61	27	12:00	28.56	50.31	2.496	1090	853
2008	62	27	12:00	27.35	60.59	2.6	1093	856
2008	63	27	12:00	28	57.3	3.062	1025	802
2008	64	27	12:00	27.47	62.48	2.967	1082	847

2008	65	28	12:00	27.58	45.69	1.694	1103	863
2008	66	28	12:00	25.12	74.8	2.581	932	730
2008	67	28	12:00	22.54	69.44	3.376	551.2	431
2008	68	28	12:00	22.45	48.94	2.453	1073	840
2008	69	28	12:00	22.61	53.48	2.222	1094	856
2008	70	28	12:00	25.02	51.97	2.857	1087	851
2008	71	28	12:00	27.47	43.74	2.433	1056	827
2008	72	29	12:00	27.24	52.52	2.852	1065	834
2008	73	29	12:00	28.61	45.39	2.951	1058	828
2008	74	29	12:00	27.39	54.56	3.073	1033	809
2008	75	29	12:00	28.33	47.99	3.769	1082	847
2008	76	29	12:00	30.07	20.26	3.144	1106	866
2008	77	29	12:00	27.36	35.54	2.42	1111	870
2008	78	29	12:00	20.01	69.13	3.407	834	653
2008	79	30	12:00	23.7	39.87	2.651	1127	882
2008	80	30	12:00	29.57	20.83	2.408	1087	851
2008	81	30	12:00	28.52	43.54	3.15	1058	828
2008	82	30	12:00	26.76	51.44	3.436	982	769
2008	83	30	12:00	26.91	46.15	3.213	1024	802
2008	84	30	12:00	23.67	74.8	3.093	771	604
2008	85	30	12:00	23.62	60.66	2.333	474.5	371
2008	86	31	12:00	25.89	45.34	2.192	797	624
2008	87	31	12:00	21.9	89.5	2.346	676.9	530
2008	88	31	12:00	22.35	83.1	1.702	600.4	470
2008	89	31	12:00	22.5	64.75	3.999	982	769
2008	90	31	12:00	22.35	65.41	1.902	938	734
2008	91	31	12:00	24.26	63.78	1.994	936	733
2008	92	31	12:00	22.99	64.99	1.86	687.2	538
2008	93	32	12:00	22.44	63.86	2.51	943	738
2008	94	32	12:00	23.04	60.29	1.582	620.5	486
2008	95	32	12:00	22.87	67.63	1.462	794	622
2008	96	32	12:00	24.42	53.76	1.534	924	723
2008	97	32	12:00	23.59	65.58	3.523	820	642
2008	98	32	12:00	21.83	63.34	2.665	788	617
2008	99	32	12:00	20.22	71.7	2.379	631.3	494

Seed germination, tree growth and flowering responses of *Moringa oleifera* Lam. (Horseradish Tree) to temperature

by

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SUMMARY

As the world experiences an age of dwindling oil reserves and unstable oil prices, alternative fuel sources have to be found. One such alternative fuel source is Biodiesel. Although numerous conventional oil-yielding biodiesel crops are already being cultivated, it is the search for a biodiesel crop able to tolerate the harsh African climate while not affecting food security that is the challenge. A tree with great potential in this respect is *Moringa oleifera,* as this is a fast growing, drought tolerant and high oil-yielding tree. However prior to the establishment of commercial plantations, all cultivation aspects of this promising tree have to be understood. This research therefore aims to provide much needed insight into the effect of various temperatures/climates on the growth of this tree.

Three temperature regimes simulating different climates were chosen to assess seed germination, seedling/tree growth and flowering in conjunction with the effect of the growth regulator paclobutrazol and hardening-off treatment on trees. The chosen temperature regimes were 10/20°C, 15/25°C and 20/30°C simulating day/night temperature conditions.

While the low 10/20°C rendered slightly higher germination percentages, the high 20/30°C regime had a lower mean germination time, higher germination rate, uniformity and seedling growth rate. The high 20/30°C regime induced slight seed dormancy, however an incubation period at reduced temperatures (10-20°C) for 5-7 days should break this dormancy, which would improve seed germination percentages.

The 20/30°C temperature regime was the most favourable towards overall tree growth, as the highest values were obtained across all measured parameters. The increase in temperature resulted in significant ($P \leq 0.05$) growth rate increases of over 650% between the 10/20°C and 20/30°C and 250% between the 10/20°C and 15/25°C night/day temperature regimes. The hardening-off pre-treatment resulted in final tree height and stem diameter increases of 3.09X, 1.44X and 1.23X higher, than their non-hardened off counterparts under the 10/20°C, 15/25°C and 20/30°C temperature regimes respectively.

Leaf area expressed more volatility with an increase in the temperature regime. Frequent cycles of leaf drop and renewed flushes were prevalent at both the 15/25°C

and 20/30°C temperature treatments, as the moisture demands of the rapidly increasing leaf area exceed the water supply by the roots.

Leaves from the 10/20°C and 20/30°C treatments were on average 0.239 mm and 0.136 mm thick respectively, which is a significant (P≤0.05) reduction of 43.1% as a result of a 10°C increase in temperature. Leaves were thicker mostly due to thicker mesophyll layers, an adaptation to minimize photoinhibition especially under lower growing temperatures.

The efficacy of paclobutrazol as a growth-regulator on *M. oleifera* is temperature dependant, reducing growth at the low 10/20°C regime, while increasing growth at both the higher 15/25°C and 20/30°C regimes. This was due to the plant protection properties of paclobutrazol against the adverse environmental stresses associated with higher temperatures. Paclobutrazol might therefore be effective as a stress-alleviator in areas of high temperatures and low rainfall.

The 15/25°C regime was found to be the most favourable for both flowering and pollen viability. The absence of inflorescence induction observed at the higher 20/30°C regime, indicate that flowering should follow vernalization.

Tropical climates with temperatures of between 20-30°C are thus ideal for the cultivation of *M. oleifera*, however satisfactory growth during the hot summer months in sub-tropical climates is achievable if temperatures remain above 0°C during winter, as trees are frost tender.